

Figure 1. Illustration of the α subunit residues that can be scanned by the β -subunit carboxyterminus. The α - and β -subunit backbones are shown in dark and light gray ribbons, respectively. The β tail is shown as a black ribbon. The locations of the C_{α} carbons of cysteine substitutions that enabled efficient crosslink between the α subunit residue and the probe cysteine are shown as dark spheres. The lighter gray spheres refer to residues that gave less amounts of crosslink. The small pale spheres refer to cysteine substitutions that led to negligible amount of crosslink. Note that α -subunit residues 90, 91, and 92 appear to be too mobile to be seen in the crystal structure of hCG and the arbitrary positions of these residues shown here are intended only to emphasize their apparent abilities to be latched to the seatbelt.

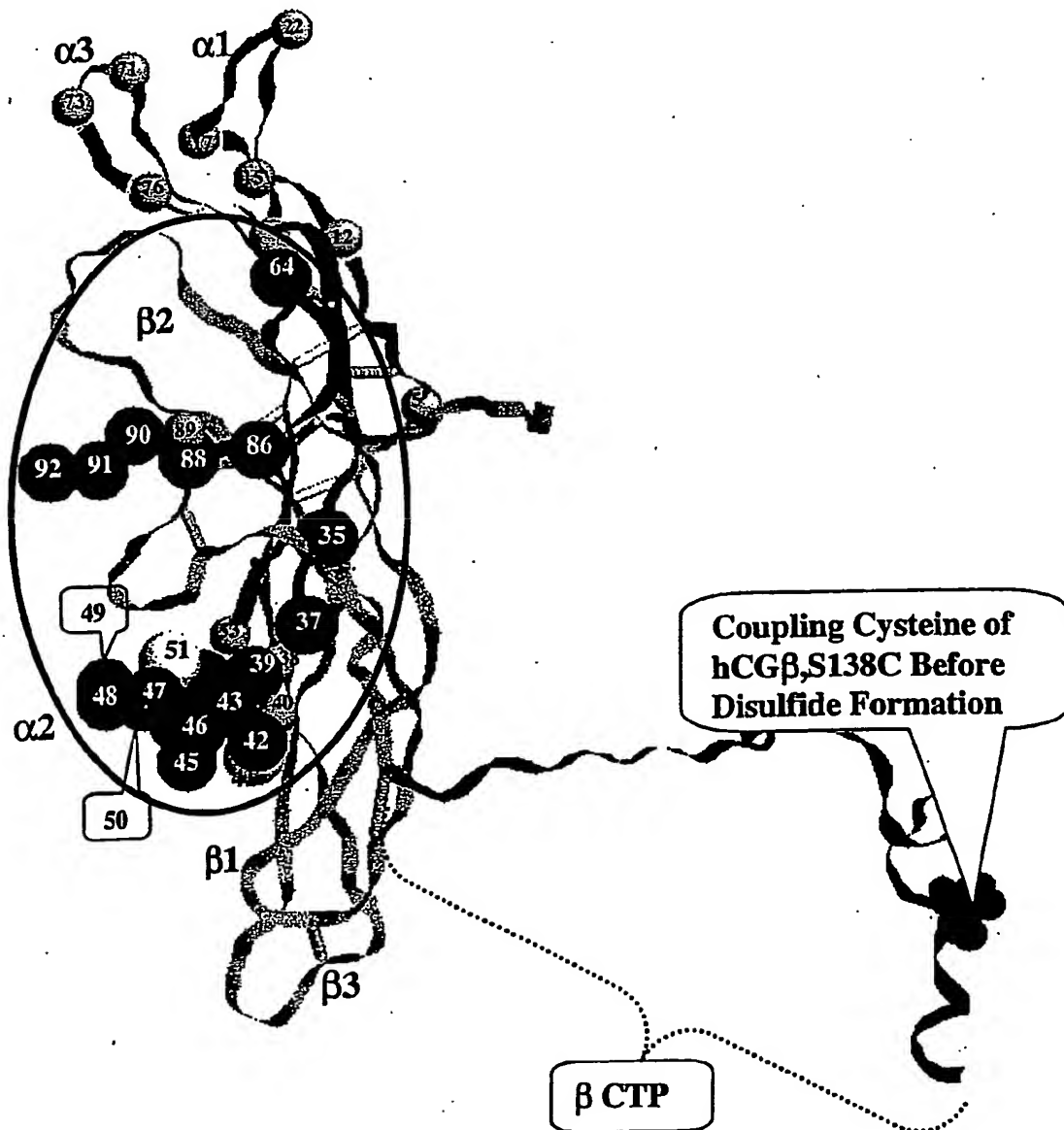


FIGURE 2

Binding of hCG or hCG analogs in which an α subunit residue in loop 2 had been substituted by a cysteine

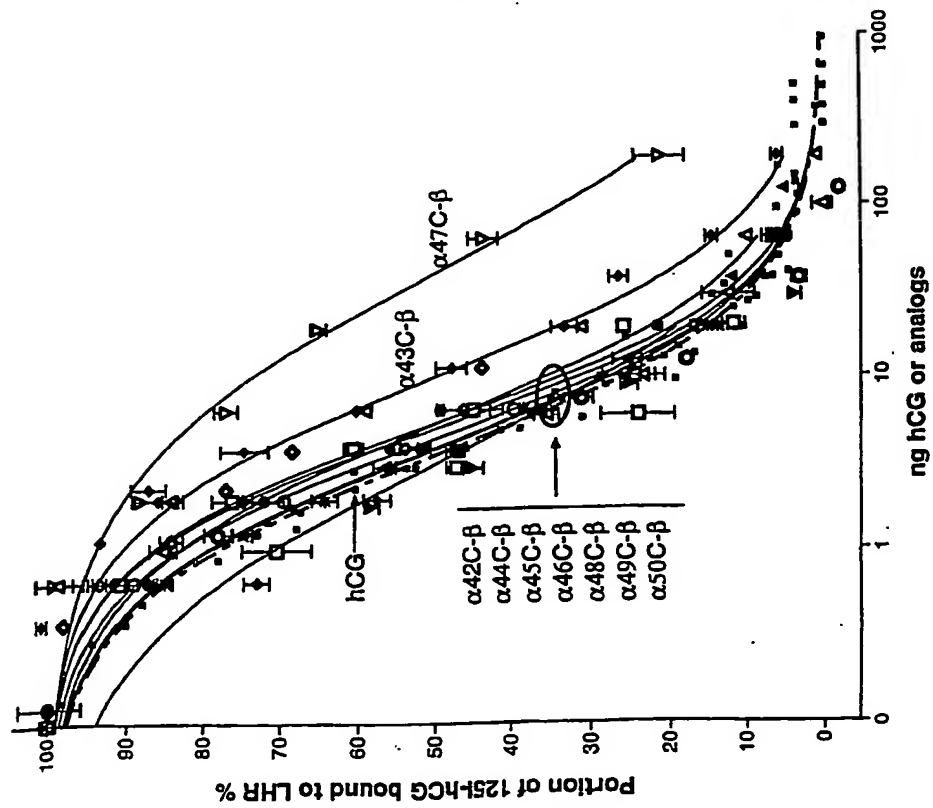


FIGURE 2A

cAMP accumulation of hCG or hCG analogs in which an α subunit residue in loop 2 had been substituted by a cysteine

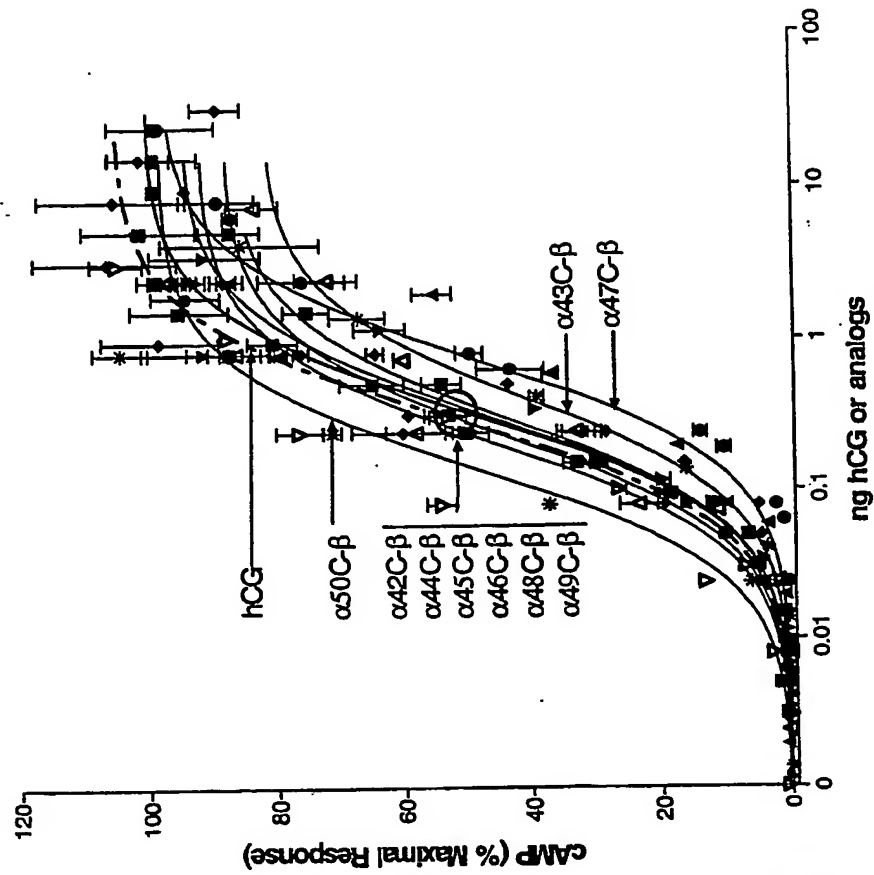


FIGURE 2B

FIGURE 3

Binding of hCG or hCG analogs in which an α subunit residue at the carboxyterminus had been substituted by a cysteine

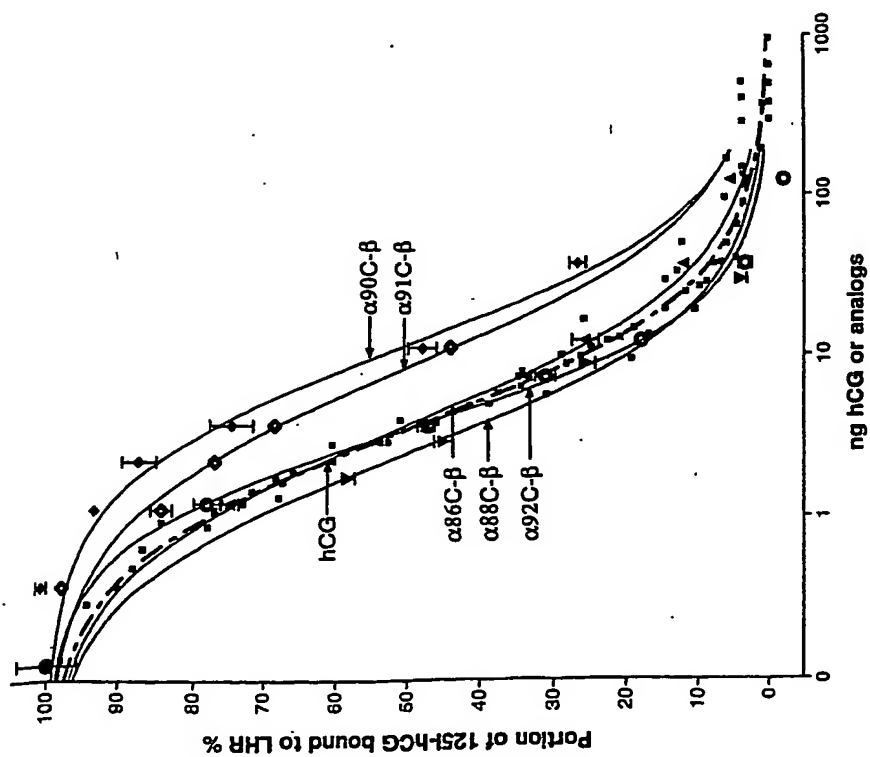


FIGURE 3A

cAMP accumulation of hCG or hCG analogs in which an α subunit residue at the carboxyterminus had been substituted by a cysteine

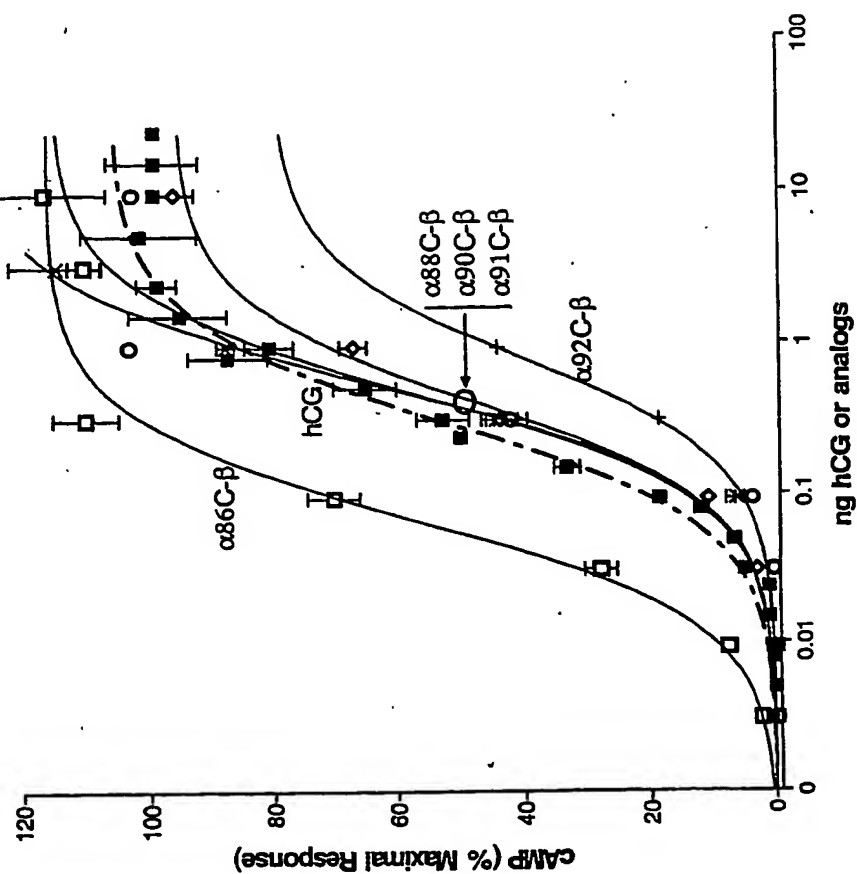


FIGURE 3B

FIGURE 4

Binding of hCG and hCG analogs with β tail attached to the α subunit residues in loop 2

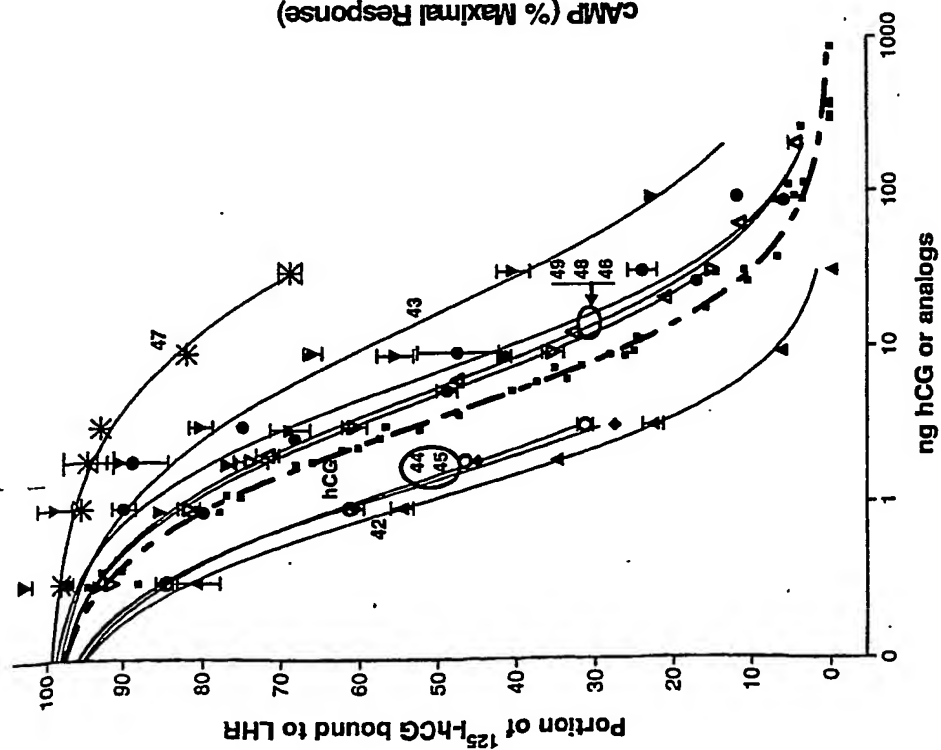


FIGURE 4A

cAMP accumulation of hCG or hCG analogs in which the β tail was attached to the α subunit residues in loop 2

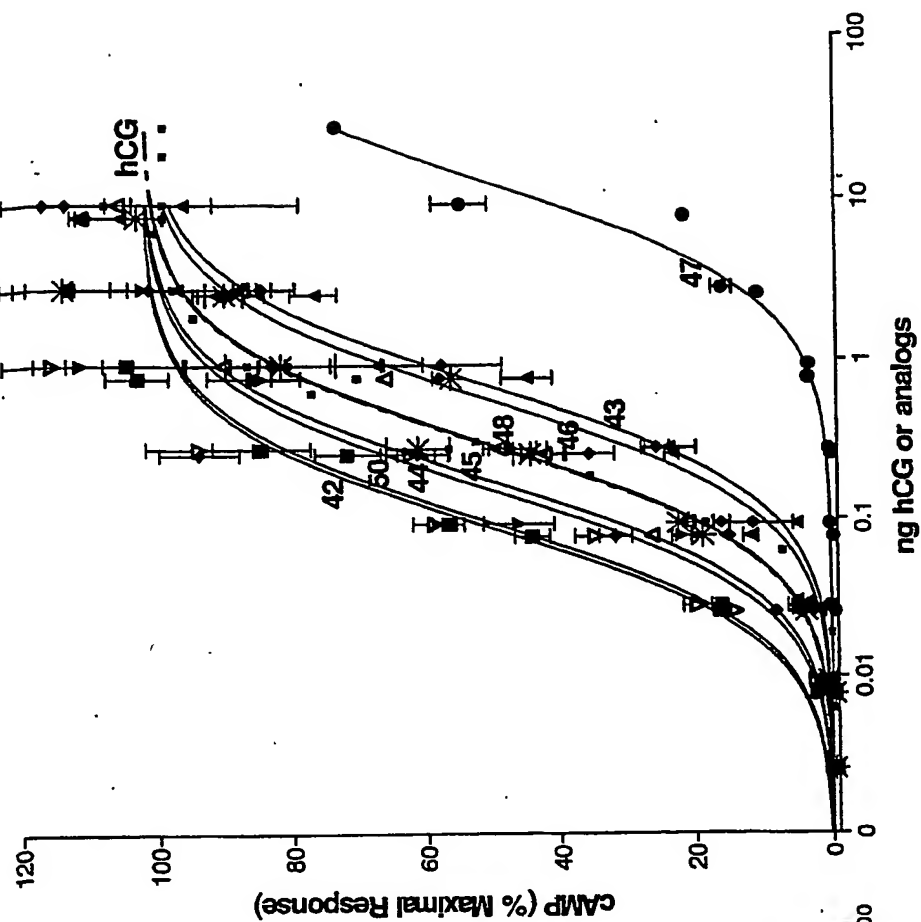


FIGURE 4B

FIGURE 5

Binding of hCG and hCG analogs with β tail attached to the α subunit residues at the carboxyterminus

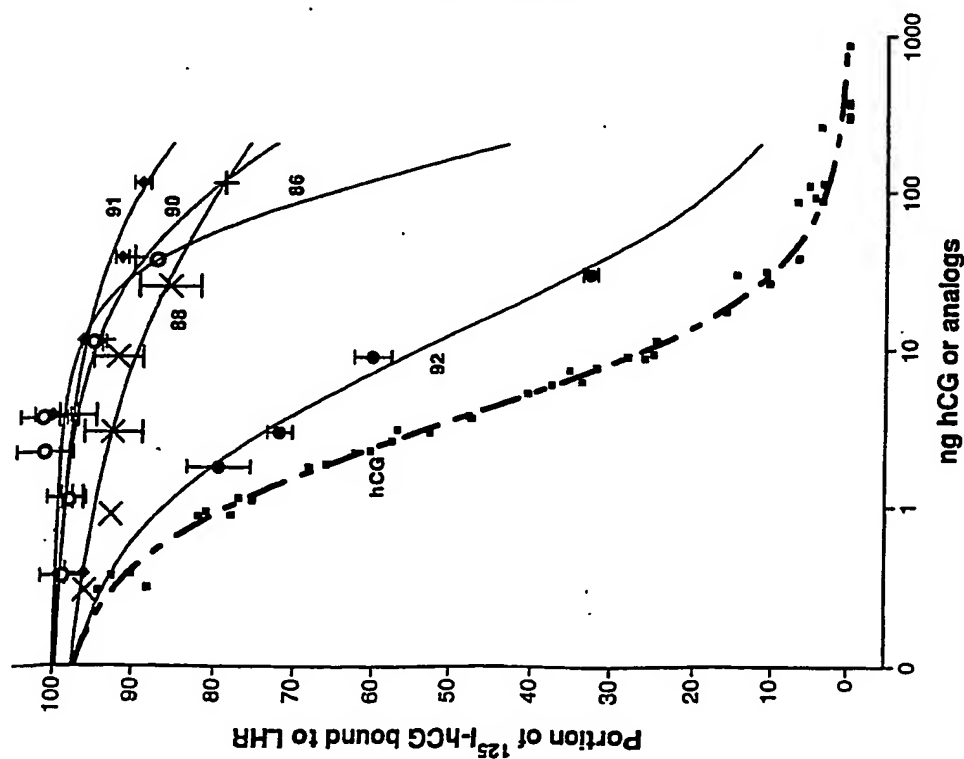


FIGURE 5A

cAMP accumulation of hCG or hCG analogs in which the β tail was attached to the α subunit residues in the carboxyterminus

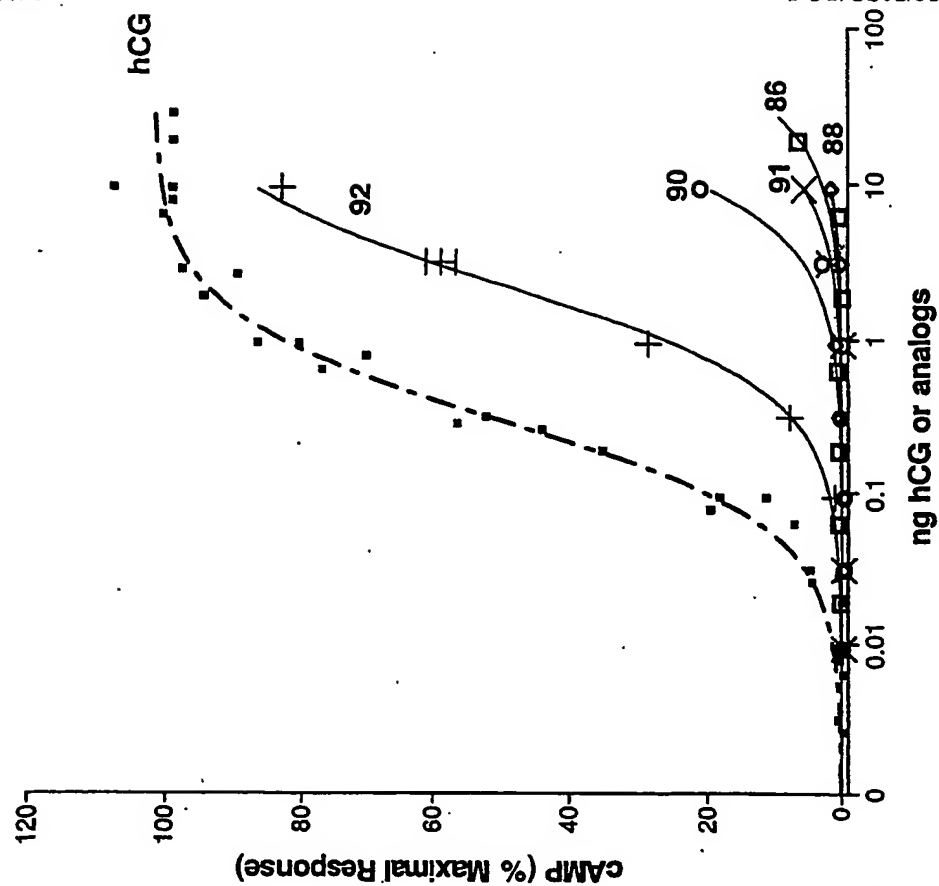


FIGURE 5B

Figure 6. Binding and signal transduction activities of the analogs in which BLA was attached to the α subunit residue.

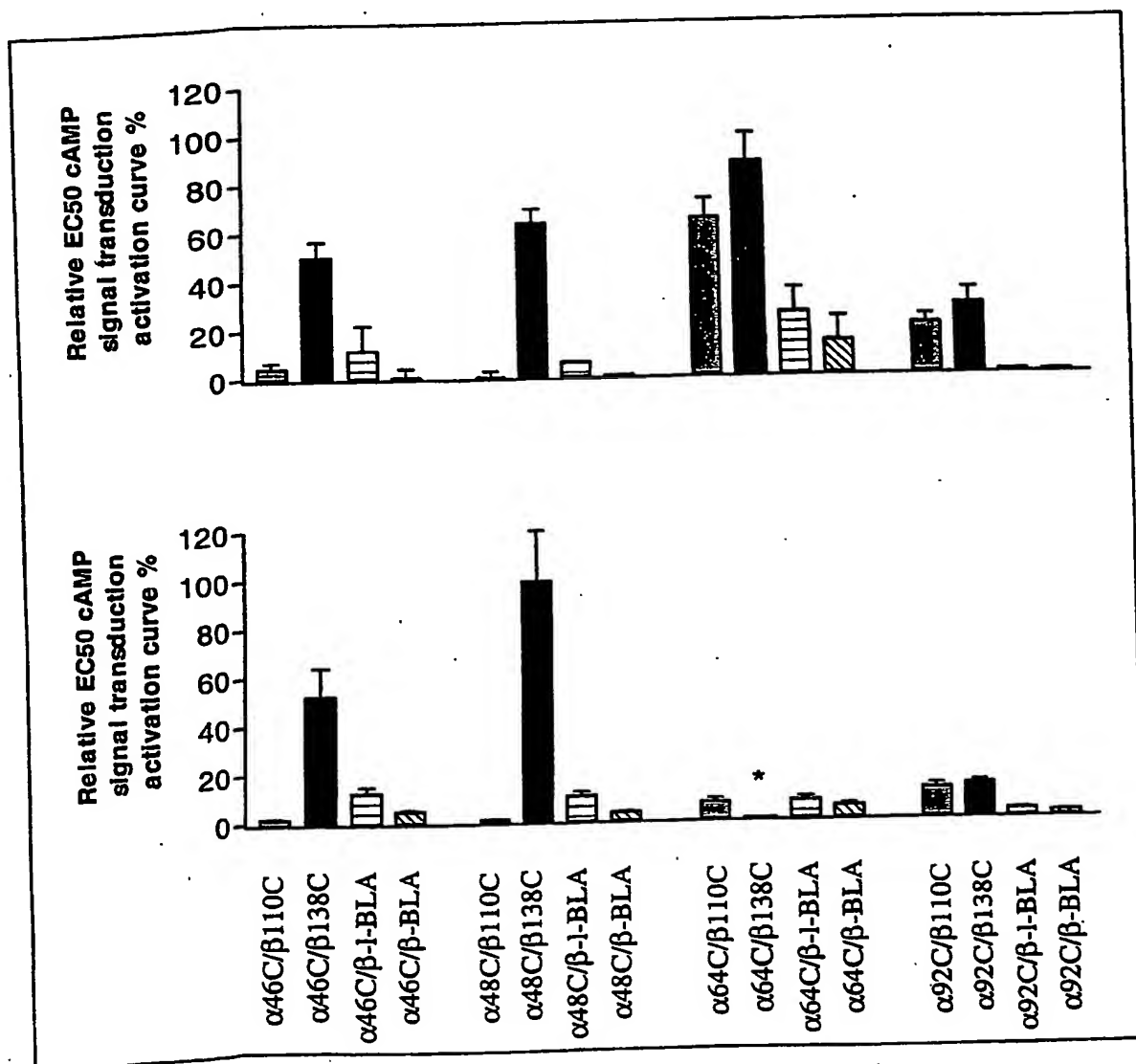


Figure 7: Amino acid sequences of the α -subunit and mutants having a substituted cysteine. (Note, the mutations are upper case and highlighted. These were prepared by standard cassette mutagenesis and PCR mutagenesis methods that are standard in the art.)

	10	20	30	40	50	60	70	80	90
α -hCG	(SEQ ID NO: 1):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α Q5C	(SEQ ID NO: 2):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α L12C	(SEQ ID NO: 3):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α N15C	(SEQ ID NO: 4):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α F17C	(SEQ ID NO: 5):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α L22C	(SEQ ID NO: 6):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α Q27C	(SEQ ID NO: 7):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α L22C	(SEQ ID NO: 8):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α R35C	(SEQ ID NO: 9):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α Y37C	(SEQ ID NO: 10):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α P38C	(SEQ ID NO: 11):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α T39C	(SEQ ID NO: 12):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α P40C	(SEQ ID NO: 13):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α L41C	(SEQ ID NO: 14):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α R42C	(SEQ ID NO: 15):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α S43C	(SEQ ID NO: 16):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α K44C	(SEQ ID NO: 17):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α K45C	(SEQ ID NO: 18):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α T46C	(SEQ ID NO: 19):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α M47C	(SEQ ID NO: 20):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α L48C	(SEQ ID NO: 21):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α V49C	(SEQ ID NO: 22):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α Q50C	(SEQ ID NO: 23):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α K51C	(SEQ ID NO: 24):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α N52C	(SEQ ID NO: 25):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α V53C	(SEQ ID NO: 26):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α E56C	(SEQ ID NO: 27):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α S64C	(SEQ ID NO: 28):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α V76C	(SEQ ID NO: 29):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α T86C	(SEQ ID NO: 30):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α Y88C	(SEQ ID NO: 31):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α L89C	(SEQ ID NO: 32):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α H90C	(SEQ ID NO: 33):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α K91C	(SEQ ID NO: 34):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							
α S92C	(SEQ ID NO: 35):	apdvqdcpectlqenpffsqpgapilqcmgcccfsrayptprskktmlvqknvtsestccvaksynrvtvmgffkvenhtachcstcyyhks							

Figure 8: Amino acid sequences of the β -subunit analogs. (Note, the substituted cysteine is in uppercase and highlighted.)

hCG β (SEQ ID NO: 36):
 10 20 30 40 50 60 70 80 90
 skeplprcrpinatlavekegcpvcitvntticagycptmtrvlqglpalpqvvcnyrdvrfesirlpgcprgvpvvsyavalscqc-
 100 110 120 130 140
 alcrrsttdcggpkdhpltdcdprfqdsssskapppslpssrlpgpsdtpilpq

hCG-S138C β (SEQ ID NO: 37):
 10 20 30 40 50 60 70 80 90
 skeplprcrpinatlavekegcpvcitvntticagycptmtrvlqglpalpqvvcnyrdvrfesirlpgcprgvpvvsyavalscqc-
 100 110 120 130 140
 alcrrsttdcggpkdhpltdcdprfqdsssskapppslpssrlpgpCtdtpilpq

CFC101-114 β (SEQ ID NO: 38):
 10 20 30 40 50 60 70 80 90
 skeplprcrpinatlavekegcpvcitvntticagycptmtrvlqglpalpqvvcnyrdvrfesirlpgcprgvpvvsyavalscqc-
 100 110 120 130 140
 alcrrsttdctvrglpsycsfgefqdsssskapppslpssrlpgpsdtpilpq

CFC101-114, S138C β (SEQ ID NO: 39):
 10 20 30 40 50 60 70 80 90
 skeplprcrpinatlavekegcpvcitvntticagycptmtrvlqglpalpqvvcnyrdvrfesirlpgcprgvpvvsyavalscqc-
 100 110 120 130 140
 alcrrsttdctvrglpsycsfgefqdsssskapppslpssrlpgpCtdtpilpq

hFSH β (SEQ ID NO: 40):
 10 20 30 40 50 60 70 80 90
 nsceltnitiaavekegcfctinttwcagycytrdlvykdparpkigtctfkelvyetvrpvcgahhadslytypvatqchcgkcdsd-
 100 110
 stdctvrglpsycsfgemke

FC1-108 β (SEQ ID NO: 41):
 10 20 30 40 50 60 70 80 90
 nsceltnitiaavekegcfctinttwcagycytrdlvykdparpkigtctfkelvyetvrpvcgahhadslytypvatqchcgkcdsd-
 100 110 120 130
 stdctvrglpsycsfgefqdsssskapppslpssrlpgpsdtpilpq

FC1-108, S132C β (SEQ ID NO: 42):
 10 20 30 40 50 60 70 80 90
 nsceltnitiaavekegcfctinttwcagycytrdlvykdparpkigtctfkelvyetvrpvcgahhadslytypvatqchcgkcdsd-
 100 110 120 130
 stdctvrglpsycsfgefqdsssskapppslpssrlpgpCtdtpil

Figure 9.

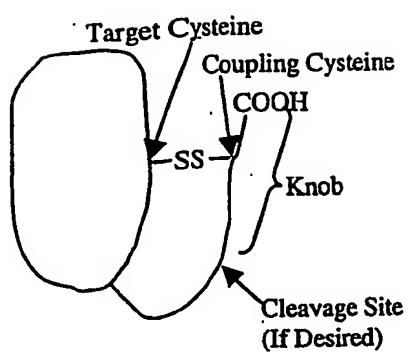


Figure 9A

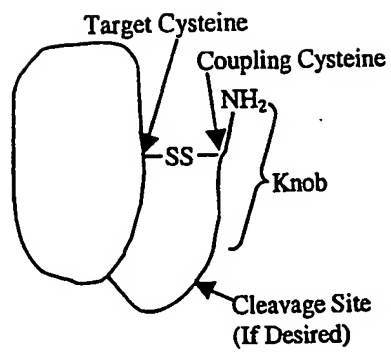


Figure 9B

Figure 10.

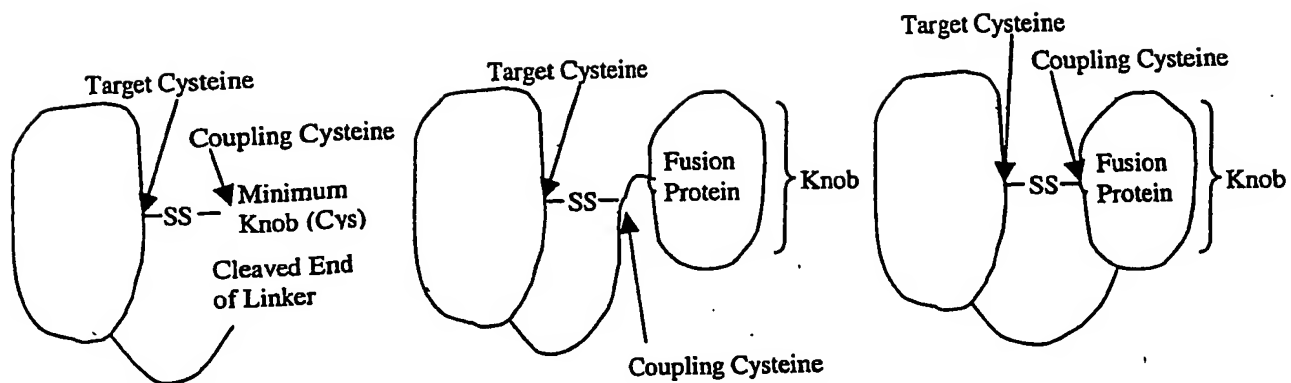


Figure 10A

Figure 10B

Figure 10C

Figure 11.

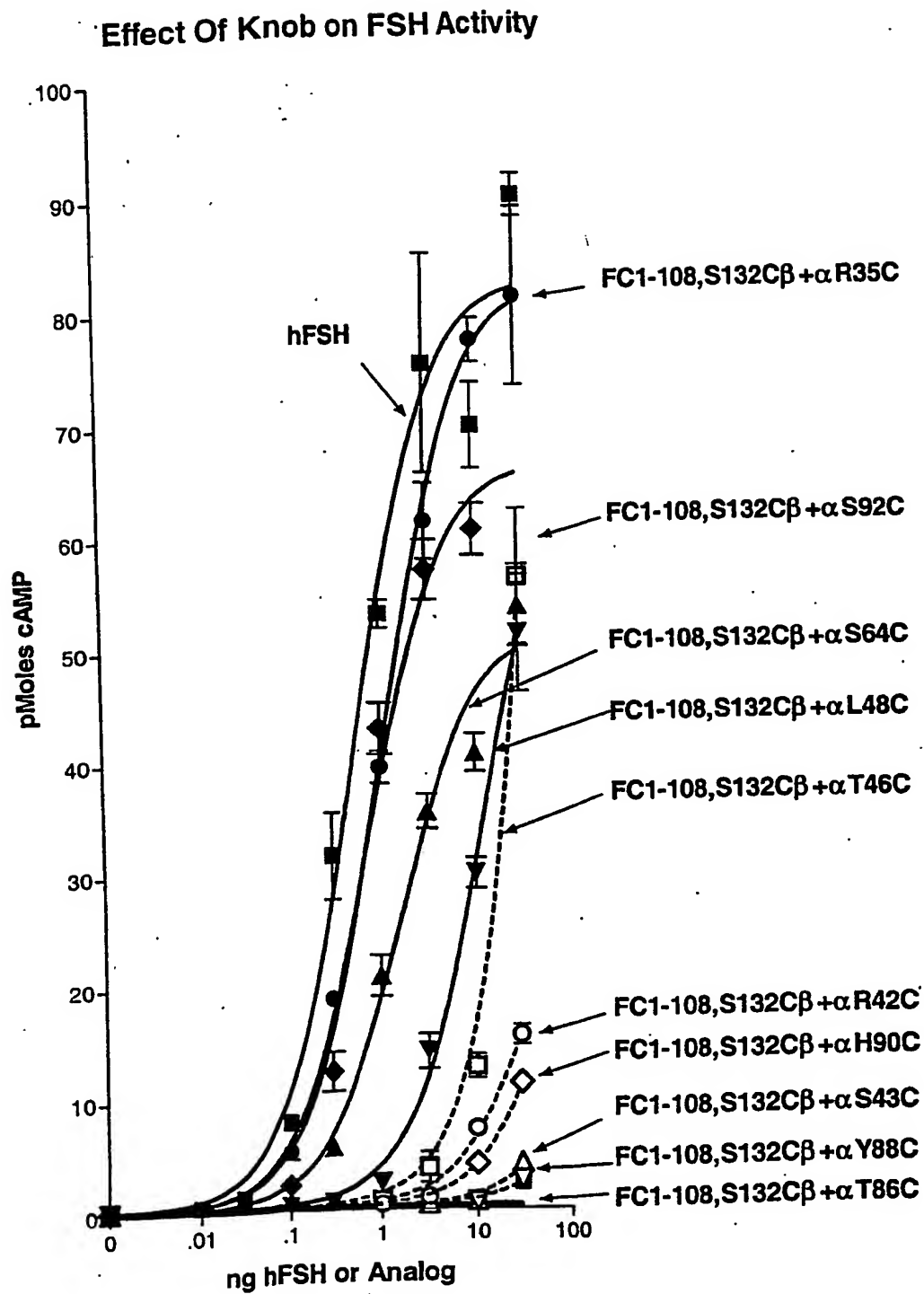


Figure 12

**Summary of Activity of Crosslinked Chimera
Analogues in LHR and FSHR Assays Relative to
CF101-109, a Bifunctional Chimera With No β CTP**

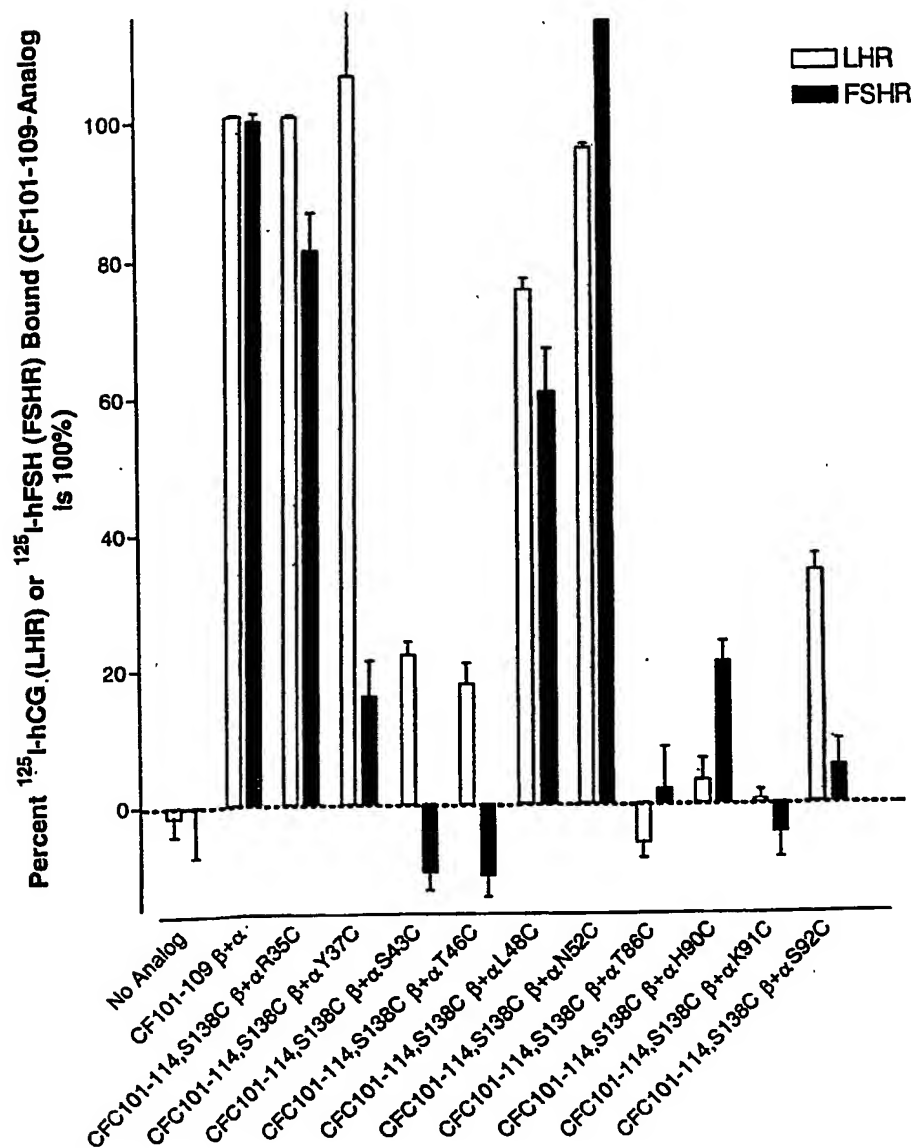


Figure 13

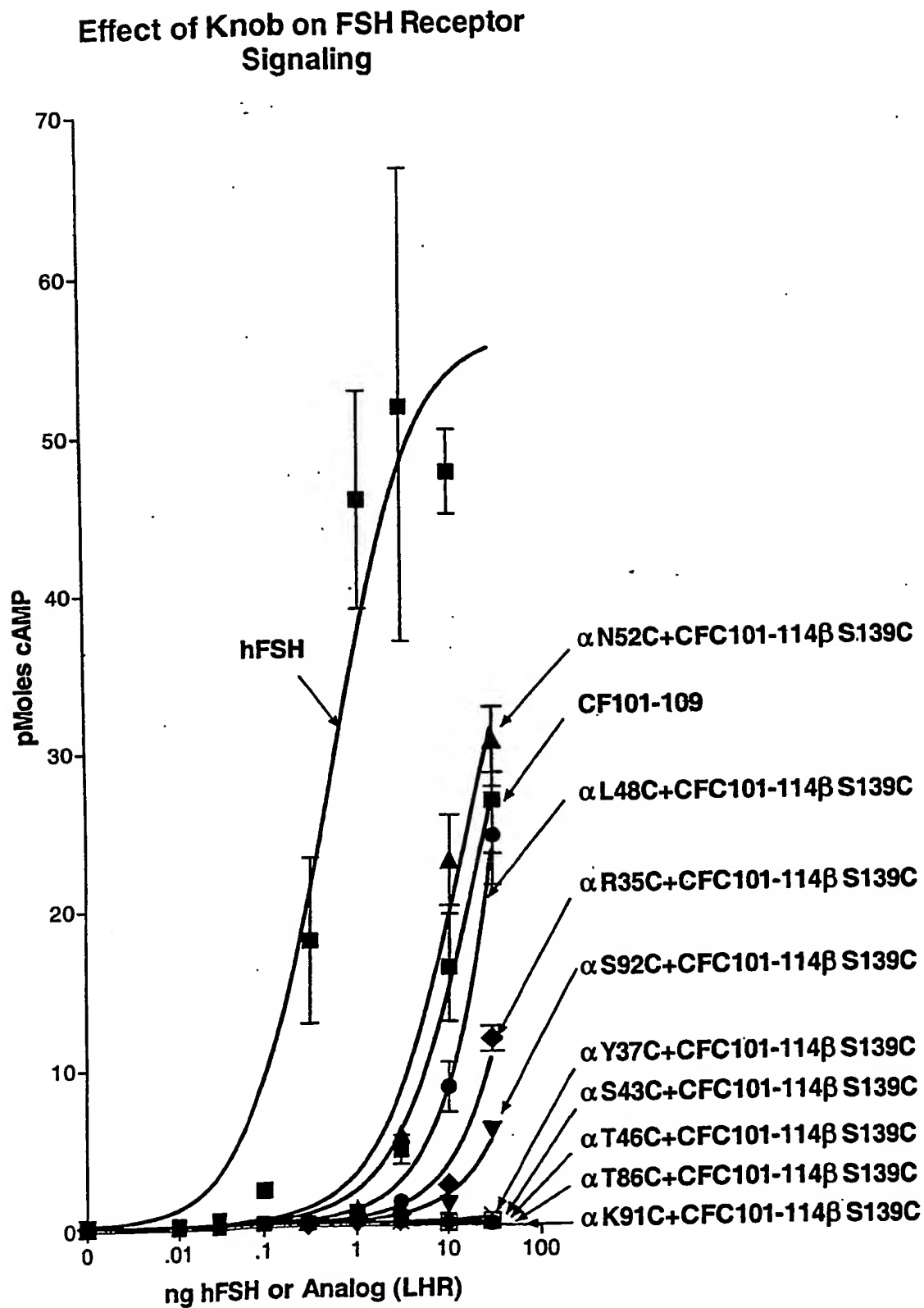


Figure 14

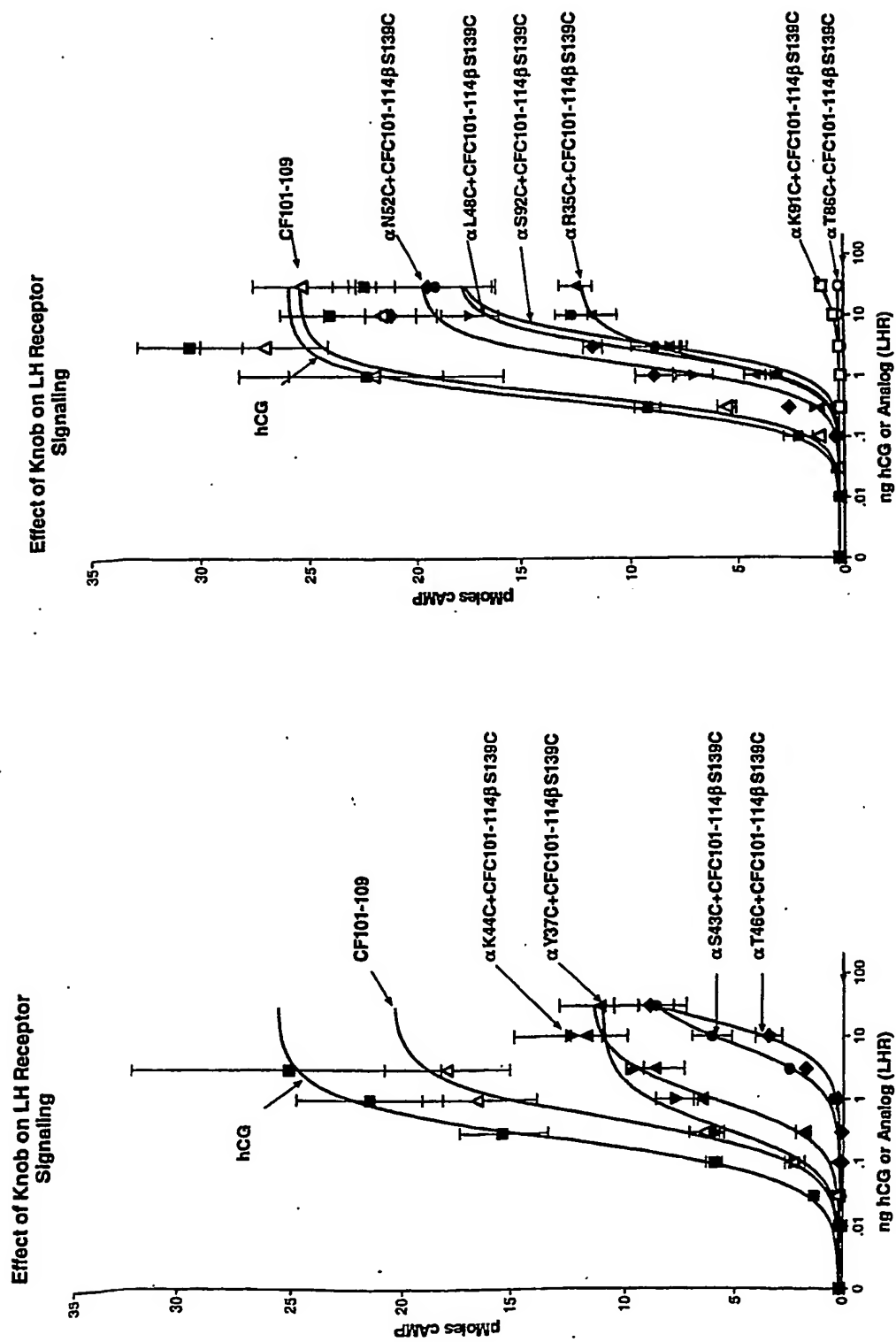


Figure 14B

Figure 14A

Figure 15: Amino acid sequences of other analogs:

hCG β .S138C- β LA(short) (SEQ ID NO: 43):

```

10      20      30      40      50      60      70      80      90
skeplrprrcrpinatlavekegcpvcitvntticagycptmtrvlqgvlpalpqvvcnyrdvrfesirlpgcprgvpvsvavalscqc-
100    110    120    130    140    150    160    170    180
alcrsttdcggpkdhpltcddprfqdsssskappspslpgpcdhpetlvkvkdaedqlgarvgvieldlnsgkilesfirpeer-
190    200    210    220    230    240    250    260    270
fpmmtsfkvlcgvlsridaggeqlgrrihysqndlveyspvtekhltcdgmtvrelcsaaitsmdntaanlllttiggpkeltaflhn-
280    290    300    310    320    330    340    350    360
mgdhvtrldrwepelneaiponderdtmpvamattlrklgtgelltlasrqqlidwmeadkvagpllrsalpagwfiadksgagergsr-
370    380    390    400
giiaalgpdgkpsrivviyttgsgatmdernrqiaieigaslikhw

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hCG β .S138C- β LA(long) (SEQ ID NO: 44):

```

10      20      30      40      50      60      70      80      90
skeplrprrcrpinatlavekegcpvcitvntticagycptmtrvlqgvlpalpqvvcnyrdvrfesirlpgcprgvpvsvavalscqc-
100    110    120    130    140    150    160    170    180
alcrsttdcggpkdhpltcddprfqdsssskappspslpgpcdhpetlvkvkdaedqlgarvgvieldlnsgkiles-
190    200    210    220    230    240    250    260    270
firpeerfpmmtsfkvlcgvlsridaggeqlgrrihysqndlveyspvtekhltcdgmtvrelcsaaitsmdntaanlllttiggpkel-
280    290    300    310    320    330    340    350    360
taflnmgdhvtrldrwepelneaiponderdtmpvamattlrklgtgelltlasrqqlidwmeadkvagpllrsalpagwfiadksga
370    380    390    400    410
gergsrgiiaalgpdgkpsrivviyttgsgatmdernrqiaieigaslikhw

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hCG β .S116-135.S138C (SEQ ID NO: 45):

```

10      20      30      40      50      60      70      80      90
skeplrprrcrpinatlavekegcpvcitvntticagycptmtrvlqgvlpalpqvvcnyrdvrfesirlpgcprgvpvsvavalscqc-
100    110    120
alcrsttdcggpkdhpltcddprfgpcdtpilpq

```

Figure 15 (cont'd.)

hCG β , δ 121-135,S138C (SEQ ID NO: 46):

10	20	30	40	50	60	70	80	90
skeplrprcrpinatlavekegcpvcitvntticagycptmtrvlqgvlpalpqvvcnyrdvrfesirlpgcprgvpvvsyavalscqc-								
100	110	120	130					
alcrsttdcggpkdhpltcddprfqdssskappppgcdtpilpq								

hCG β , δ 126-135,S138C (SEQ ID NO: 47):

10	20	30	40	50	60	70	80	90
skeplrprcrpinatlavekegcpvcitvntticagycptmtrvlqgvlpalpqvvcnyrdvrfesirlpgcprgvpvvsyavalscqc-								
100	110	120	130					
alcrsttdcggpkdhpltcddprfqdssskappppgcdtpilpq								

hCG β , δ 131-135,S138C (SEQ ID NO: 48):

10	20	30	40	50	60	70	80	90
skeplrprcrpinatlavekegcpvcitvntticagycptmtrvlqgvlpalpqvvcnyrdvrfesirlpgcprgvpvvsyavalscqc-								
100	110	120	130	140				
alcrsttdcggpkdhpltcddprfqdssskappppgcdtpilpq								

α K91E (SEQ ID NO: 49): apdvqdcpectlqenpffsqpgapilqcmgccfsrayptplrsakttmlvqknvtsestccvaksynrvtvmggtkvenhtachcstcyyhks 90

α K91M (SEQ ID NO: 50): apdvqdcpectlqenpffsqpgapilqcmgccfsrayptplrsakttmlvqknvtsestccvaksynrvtvmggtkvenhtachcstcyyhms

α K44A (SEQ ID NO: 51): apdvqdcpectlqenpffsqpgapilqcmgccfsrayptplrsakttmlvqknvtsestccvaksynrvtvmggtkvenhtachcstcyyhks

α K44E,K45Q (SEQ ID NO: 52): apdvqdcpectlqenpffsqpgapilqcmgccfsrayptplrsakttmlvqknvtsestccvaksynrvtvmggtkvenhtachcstcyyhks

α K44R (SEQ ID NO: 53): apdvqdcpectlqenpffsqpgapilqcmgccfsrayptplrsakttmlvqknvtsestccvaksynrvtvmggtkvenhtachcstcyyhks

Figure 16

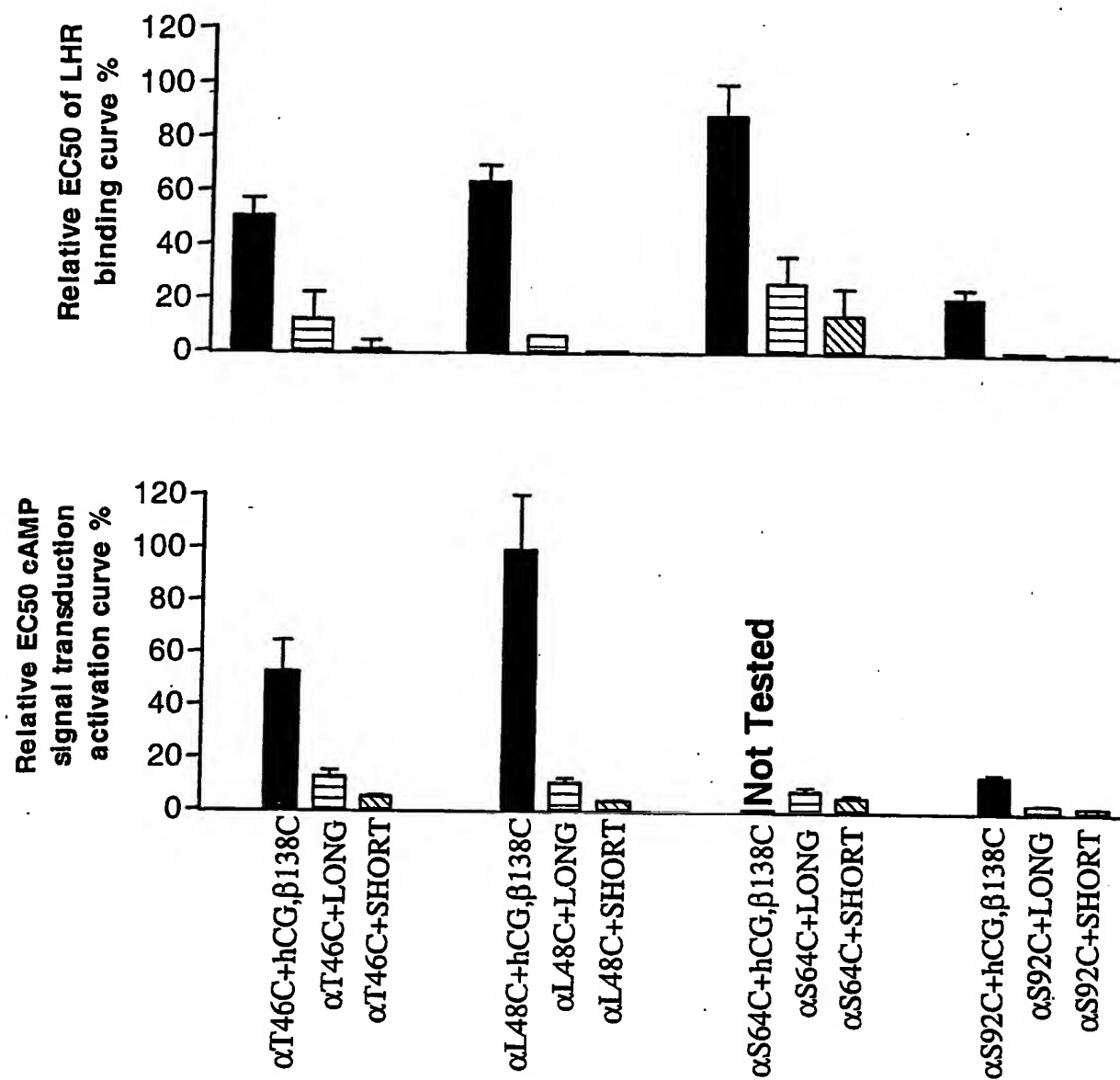


Figure 17

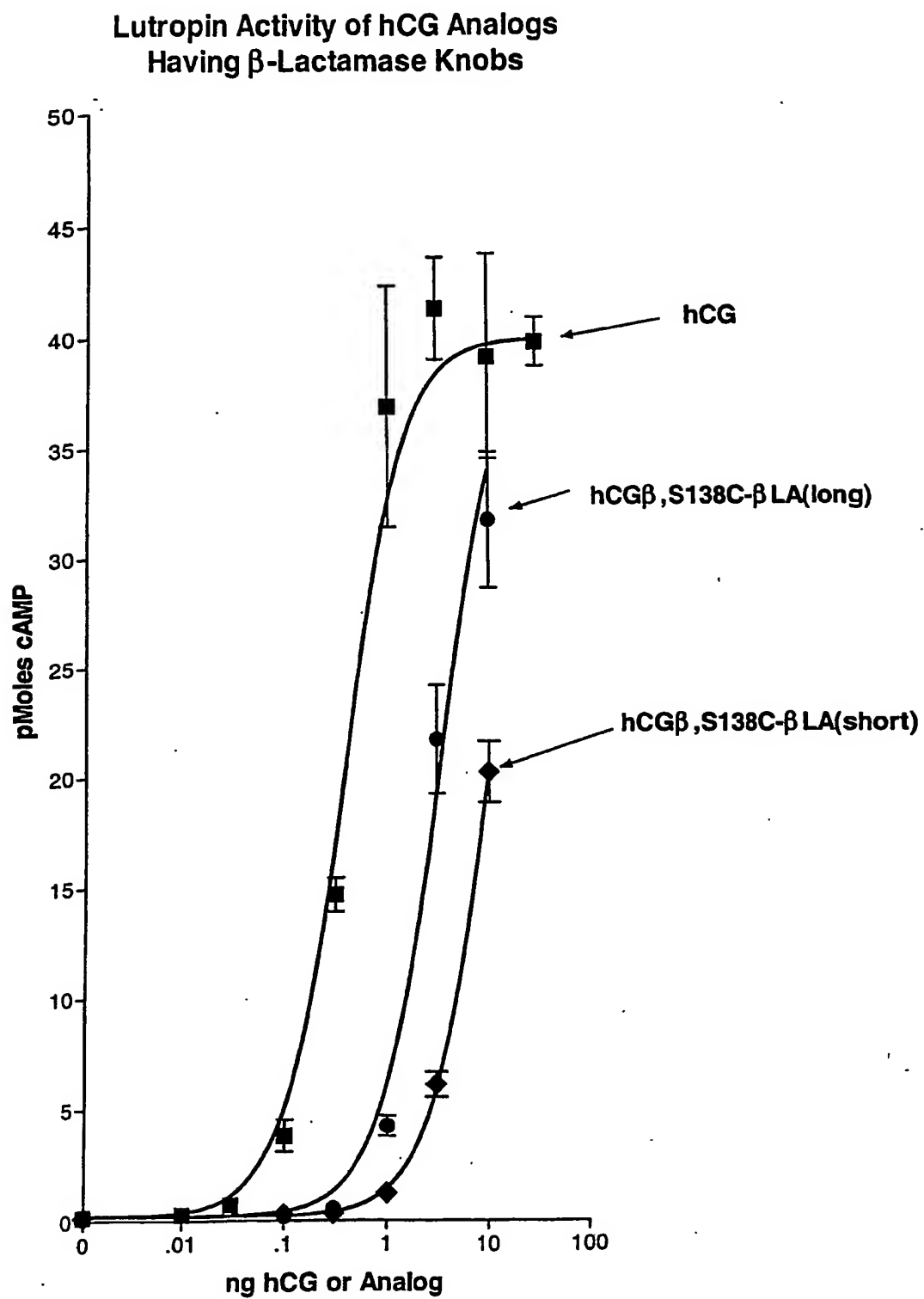


Figure 18

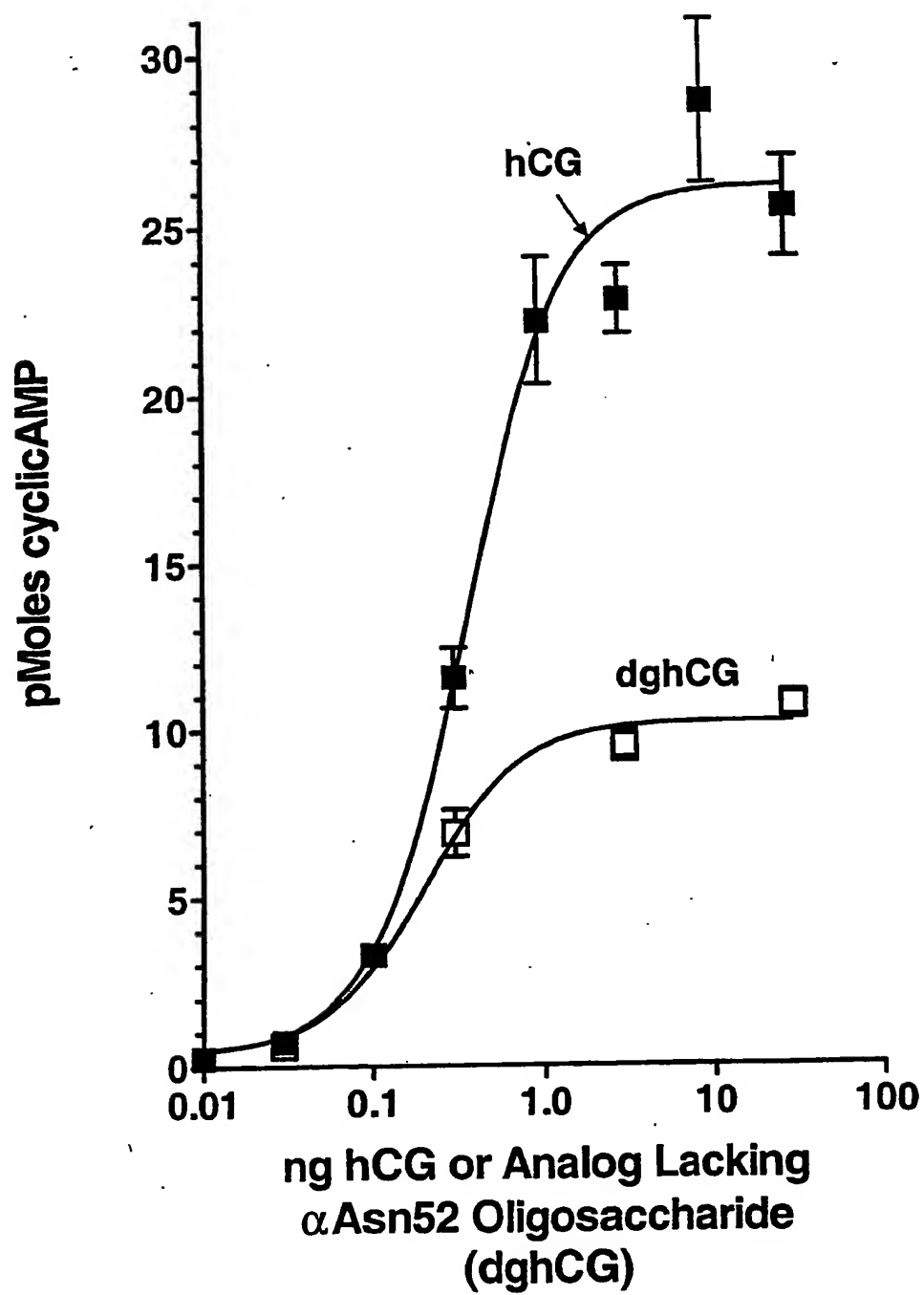
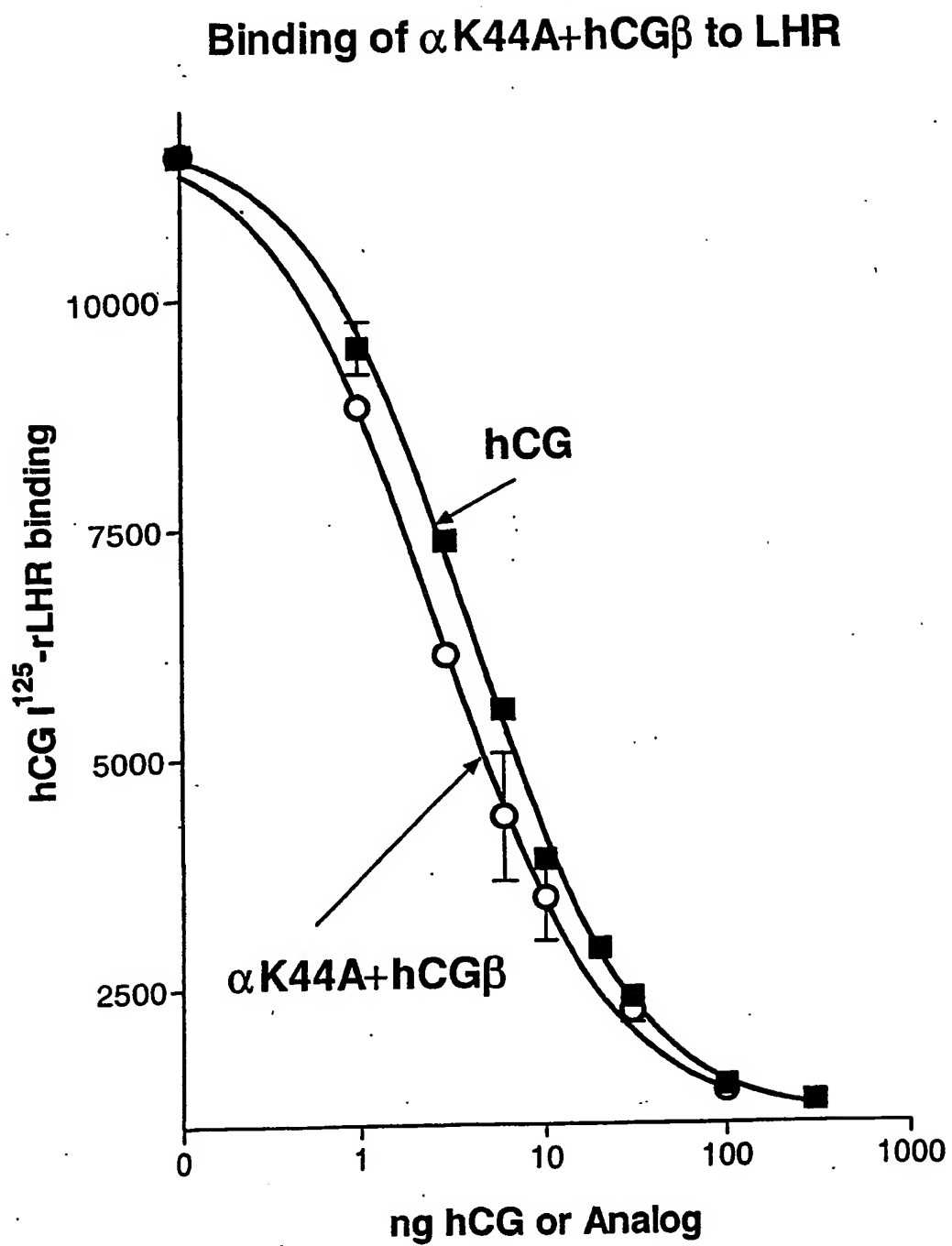


FIGURE 19



Binding of hCG and hCG Analog α K44E,K45Q+hCG β to LHR

FIGURE 20

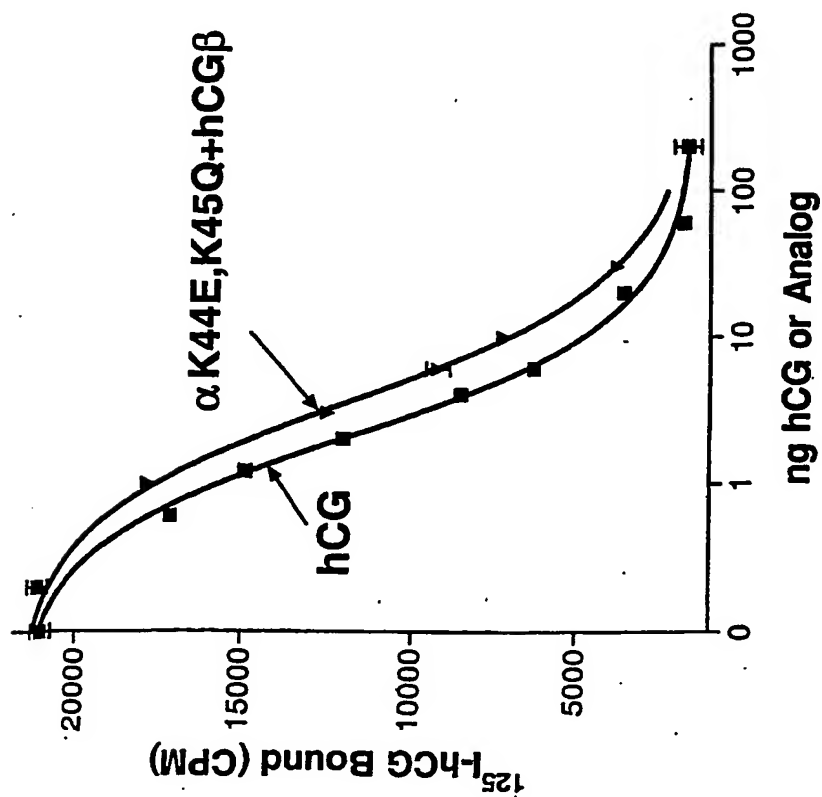


FIGURE 20A

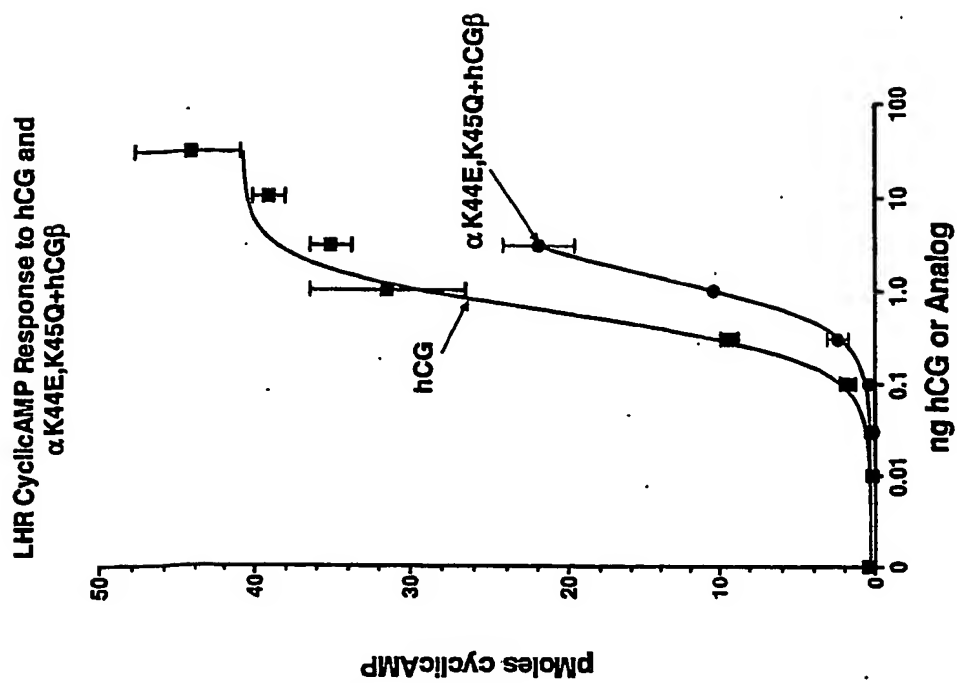


FIGURE 20B

FIGURE 21

Binding of hCG and hCG Analog
 α K91E+hCG β to LHR

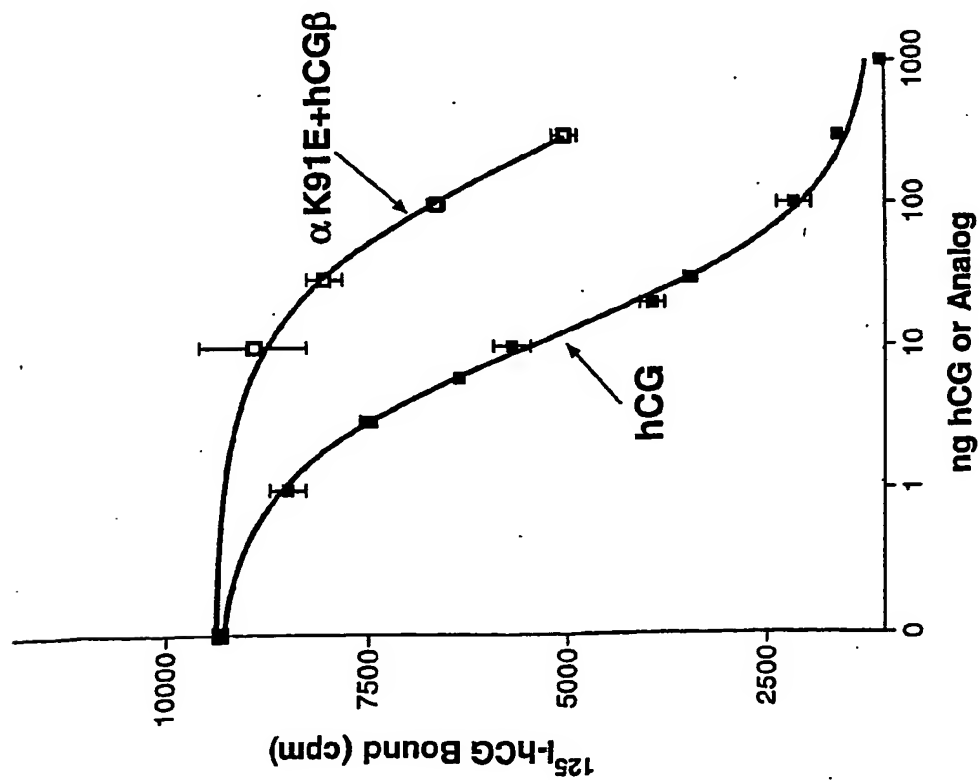


FIGURE 21A

Relative Activities of hCG and
 α K91E+hCG β in LHR cyclic AMP
Accumulation Assays

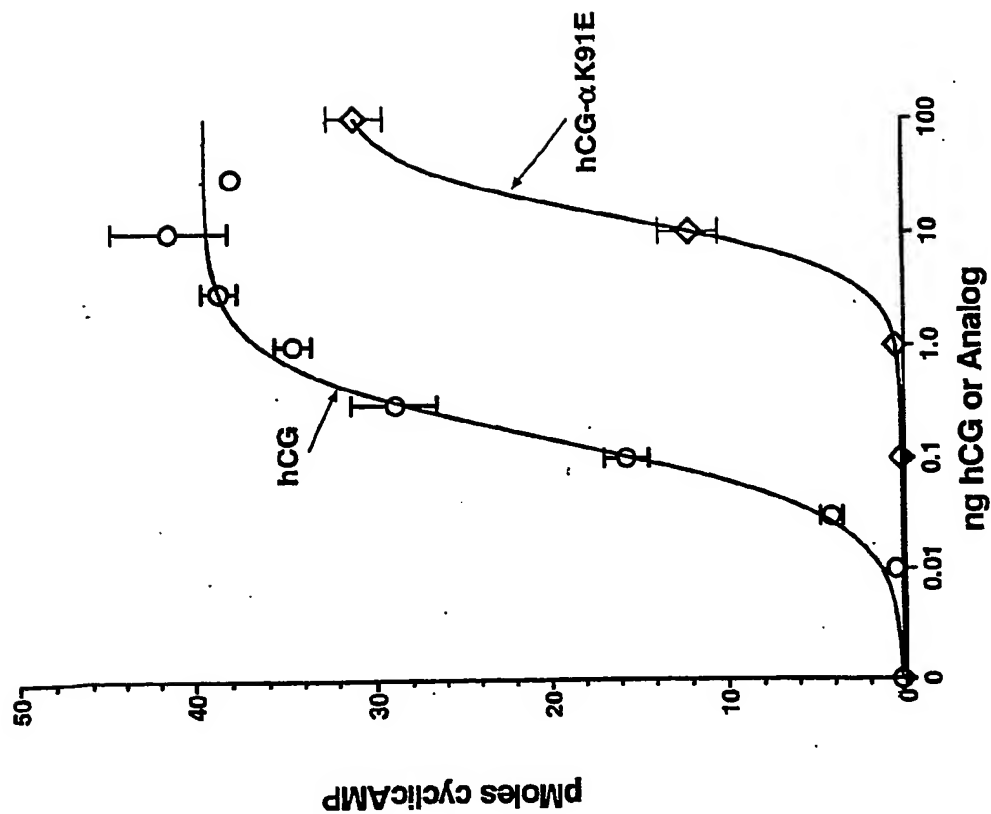


FIGURE 21B

FIGURE 22

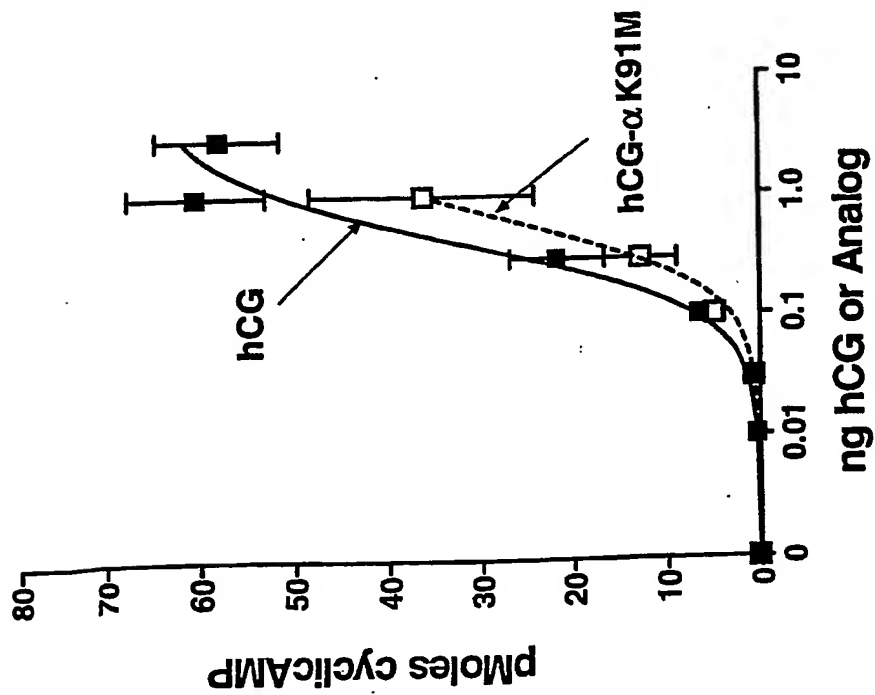
**LHR CyclicAMP Response
hCG and α K91M+hCG β** 

FIGURE 22A

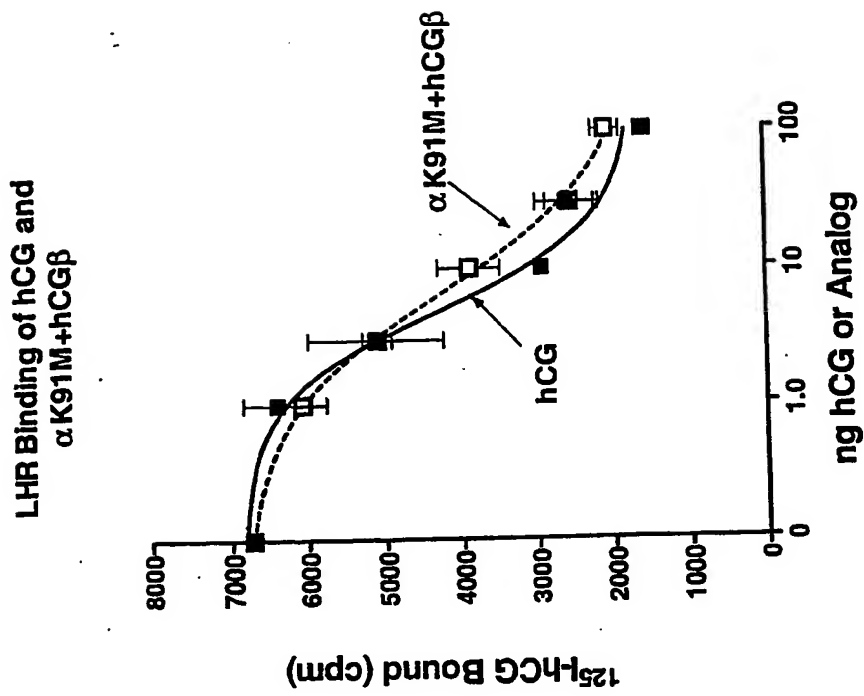


FIGURE 22B

FIGURE 23

Binding of hCG and Analogs
Containing Shortened Linkers to LHR

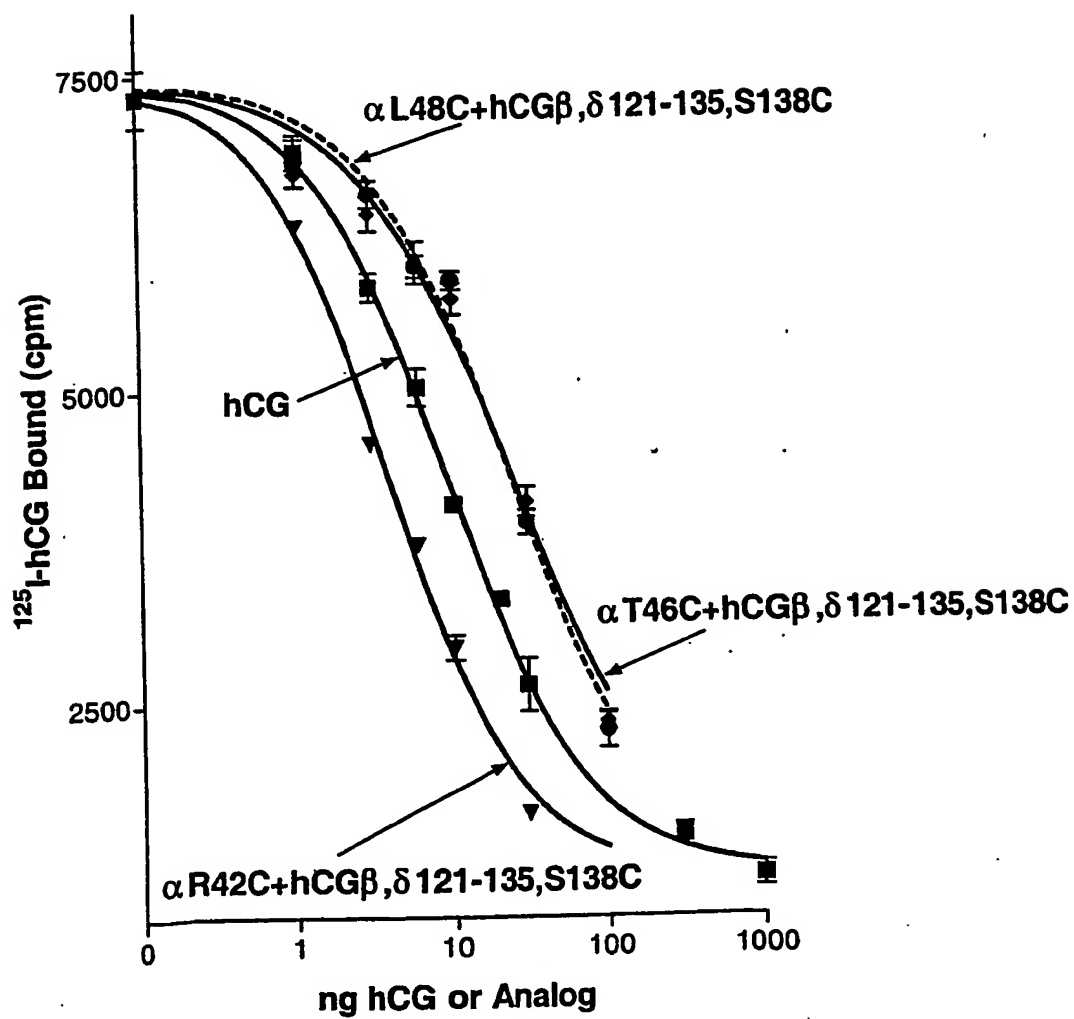


FIGURE 24

Binding of hCG and an Analog Containing a Shortened Linker to LHR

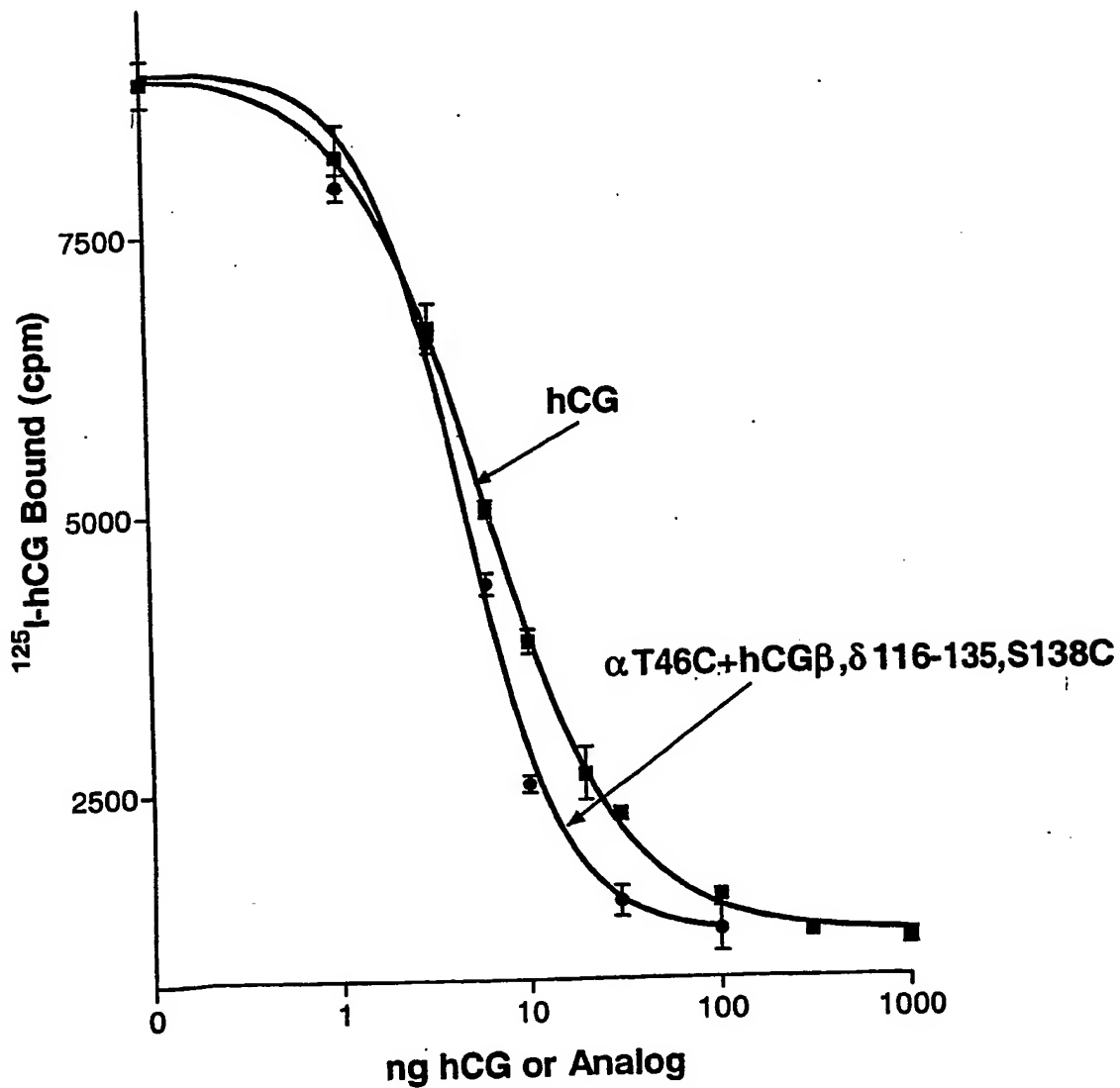
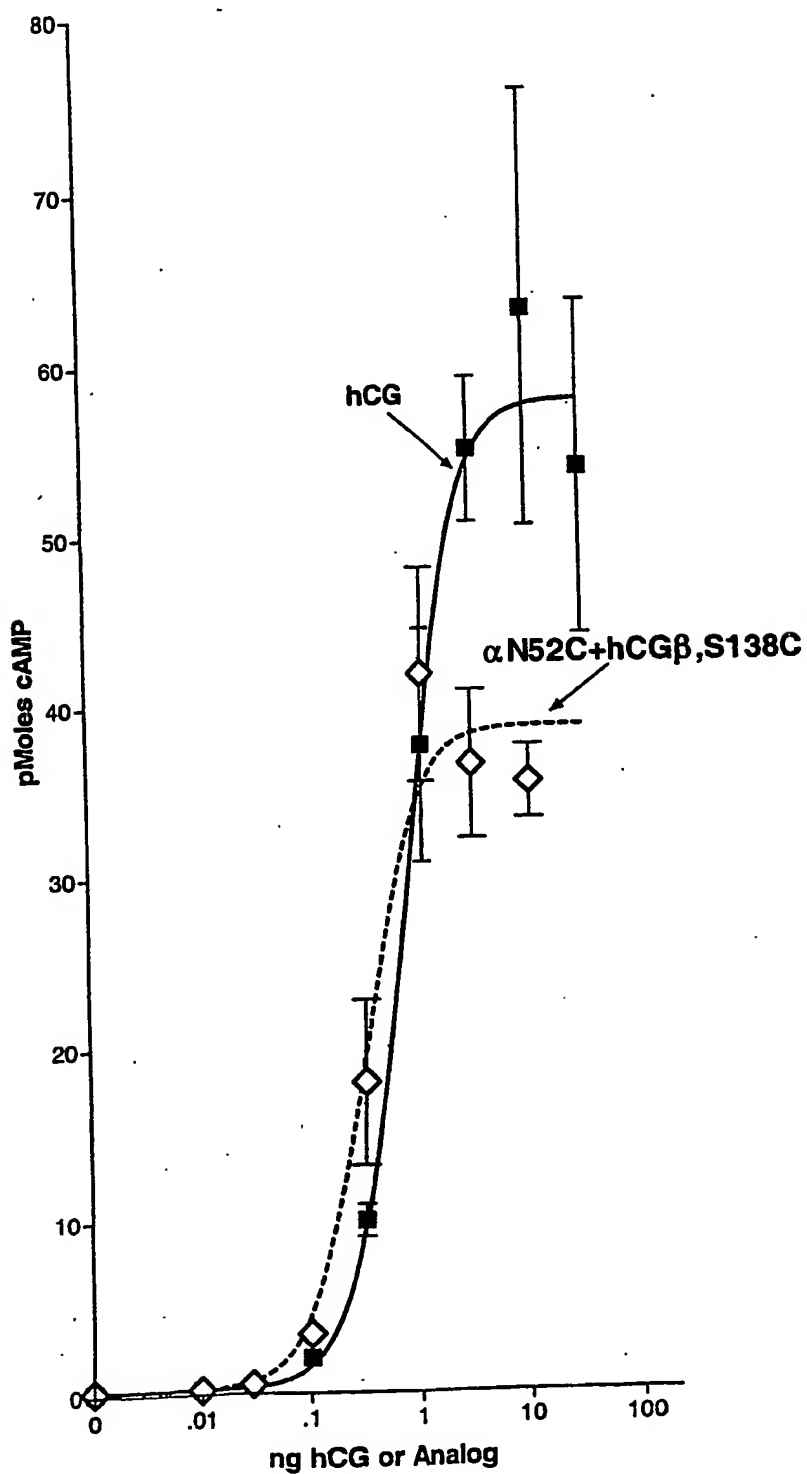


FIGURE 25

Stimulation of LHR cyclic AMP by
hCG and α N52C+hCG β ,S138C



**Binding of hCG Analogs in which a Tail Added
to the α -Subunit is Used to Add a Knob to
 β -Subunit Residues 96, 97, or 98**

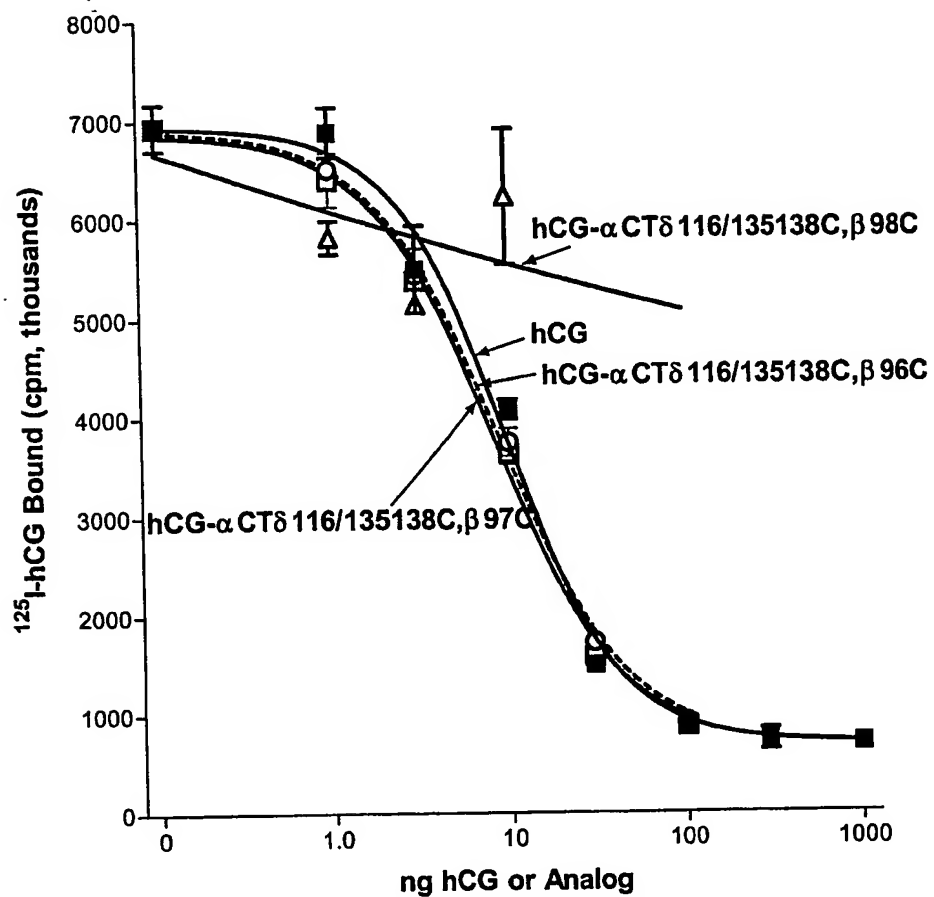


Figure 26

**Signaling of hCG Analogs in which a Truncated
Tail Added to the α -Subunit is Used to Add a
Knob to β -Subunit Residues 98 and 99**

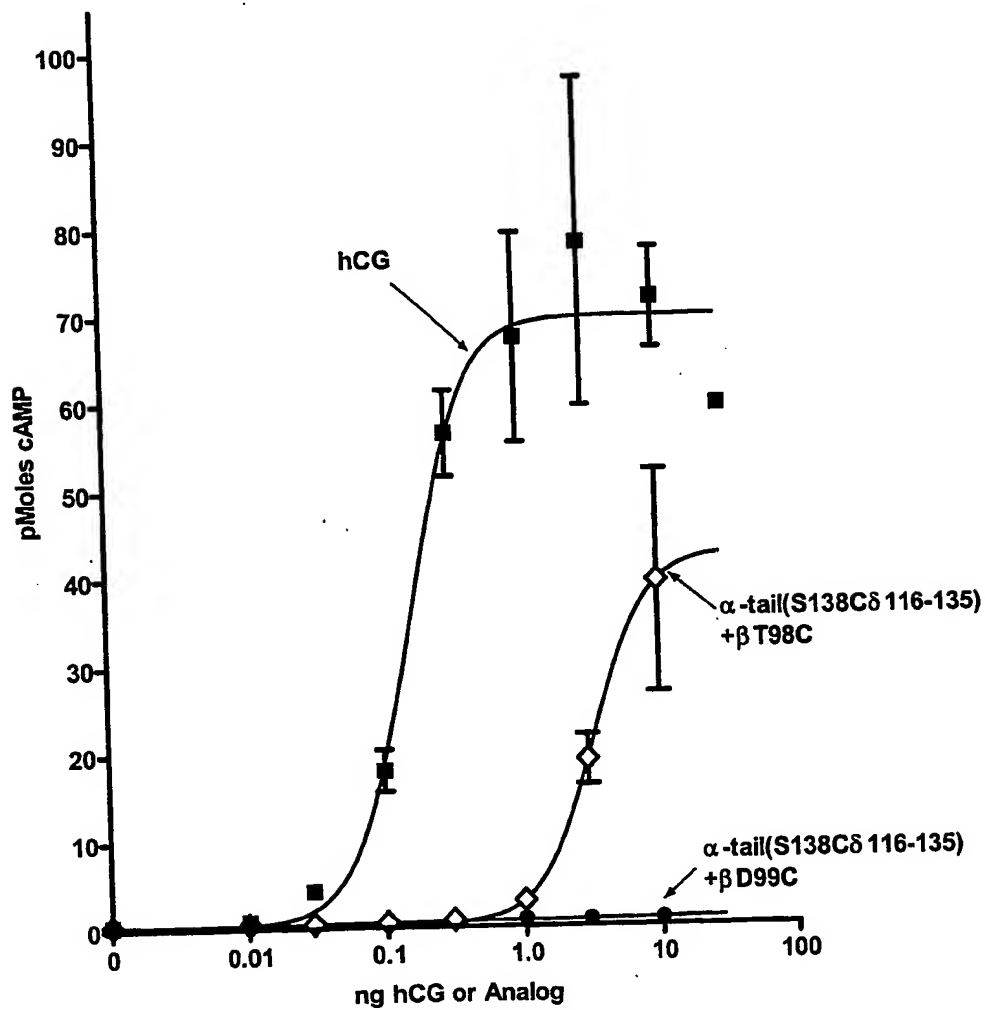


Figure 27

**Binding of hCG Analogs in which a Tail Added
to the α -Subunit is Used to Add a Knob to
 β -Subunit Residues 95 or 96**

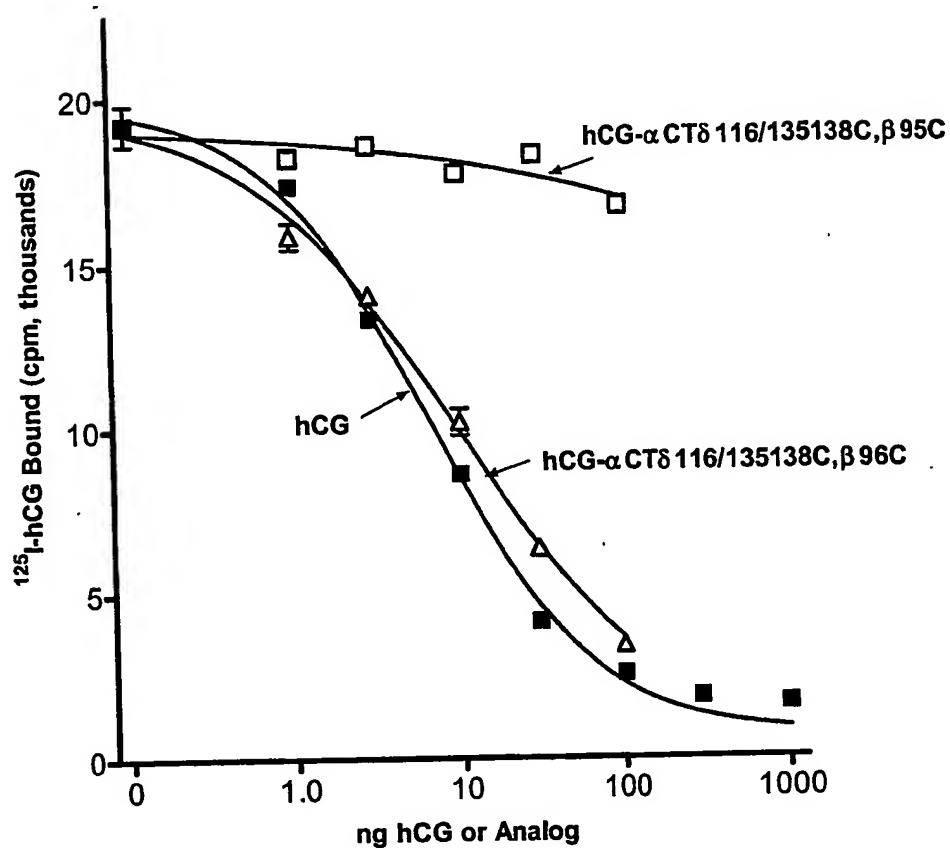


Figure 28

**Signaling of hCG Analogs in which a Tail Added
to the α -Subunit is Used to Add a Knob to
 β -Subunit Residues 95 or 96**

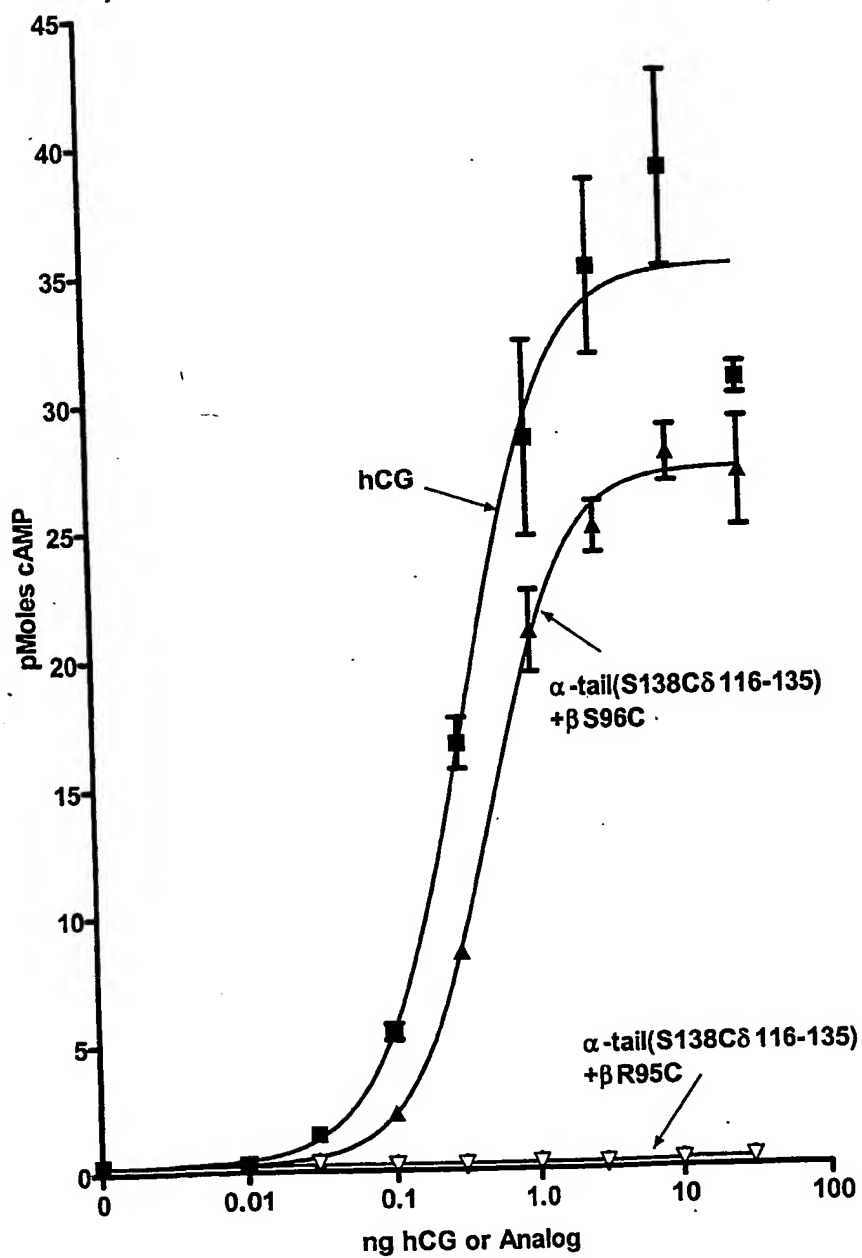


Figure 29

Signaling of hCG Analogs in which in which a GGC Tail on the α -Subunit was Used to Attach a Cysteine Knob to β -Subunit residue 96 and in which a Truncated β -Subunit Tail at the End of the α -Subunit Was used to attach a Knob to Cysteine 96 of a Bifunctional Chimera

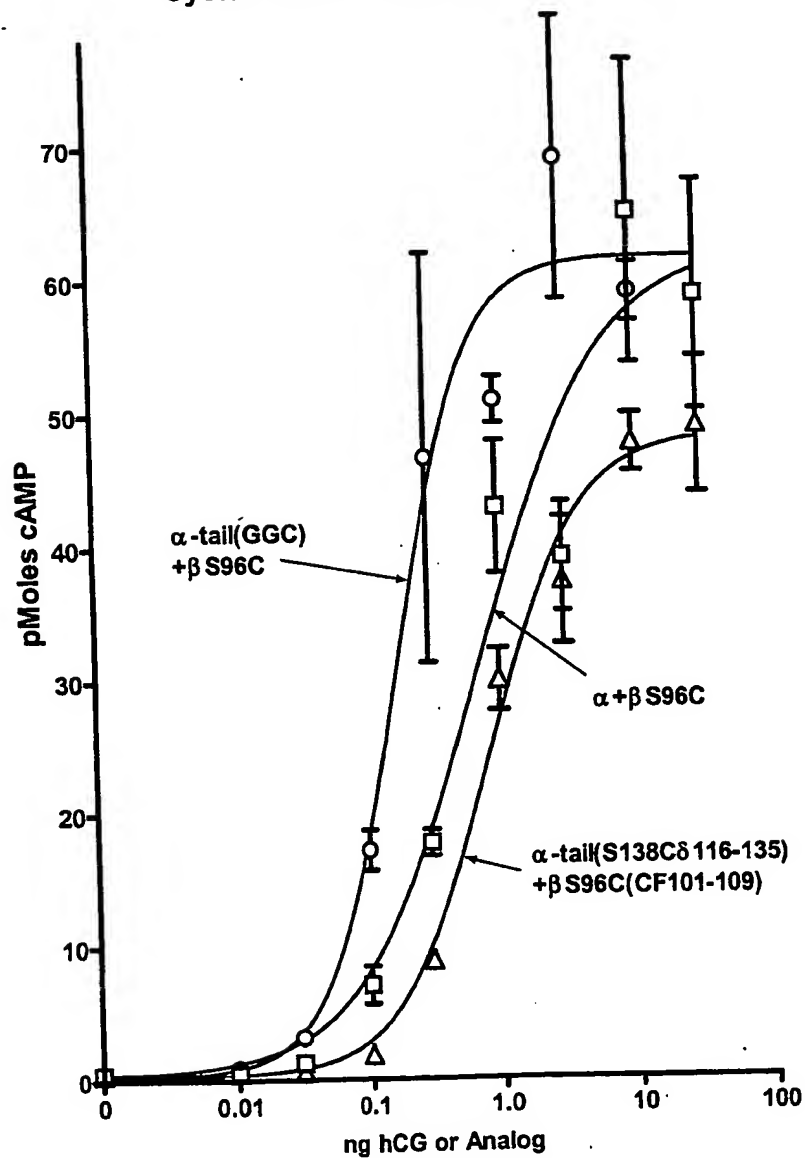


Figure 30

Signaling of hCG Analogs in which a Truncated
Tail Added to the α -Subunit is Used to Add a
Knob to β -Subunit Residues 98 or 99

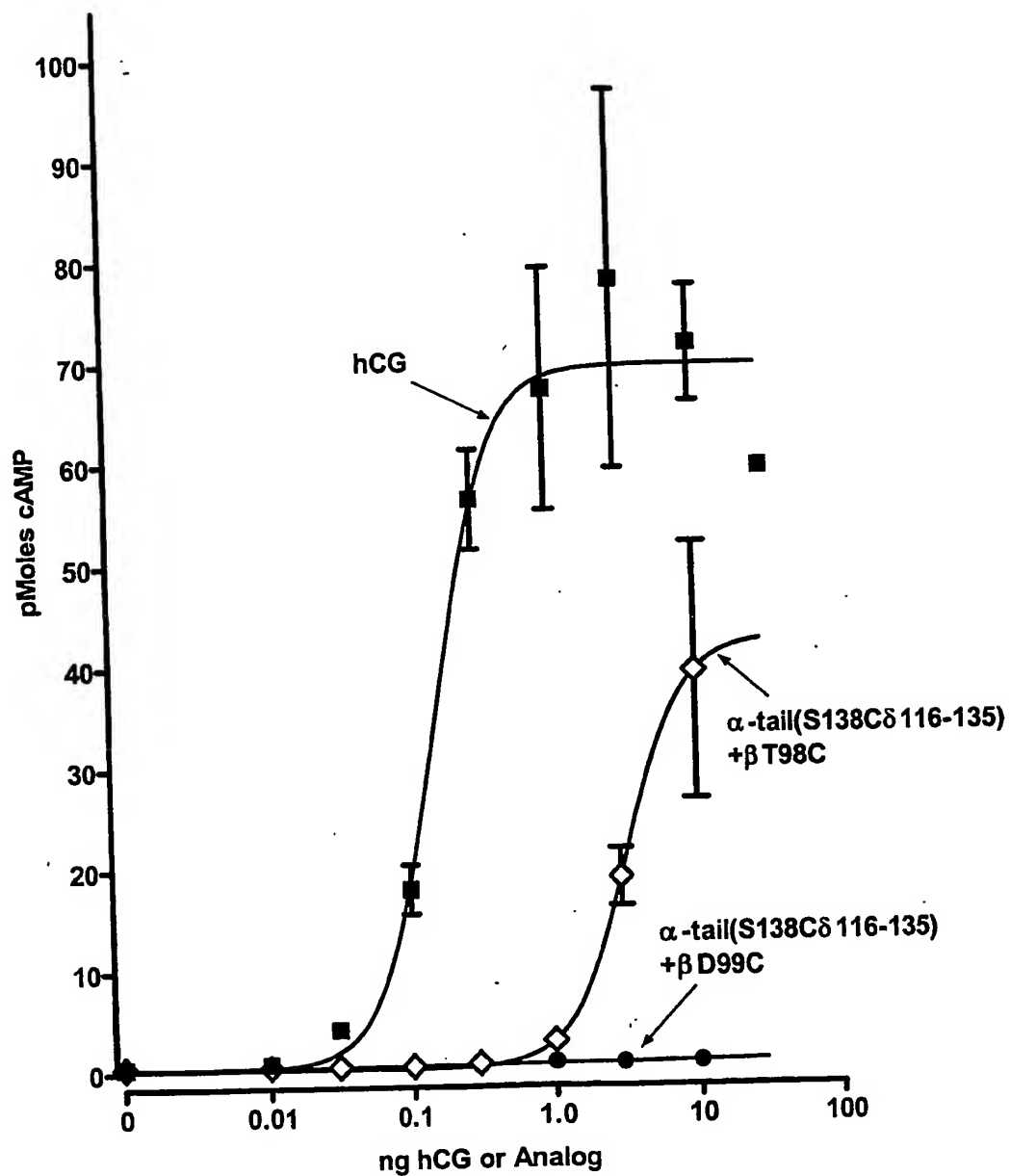


Figure 31

Influence of the Size of Knob Attached to
 β -Subunit Residue 95 on Signal Transduction

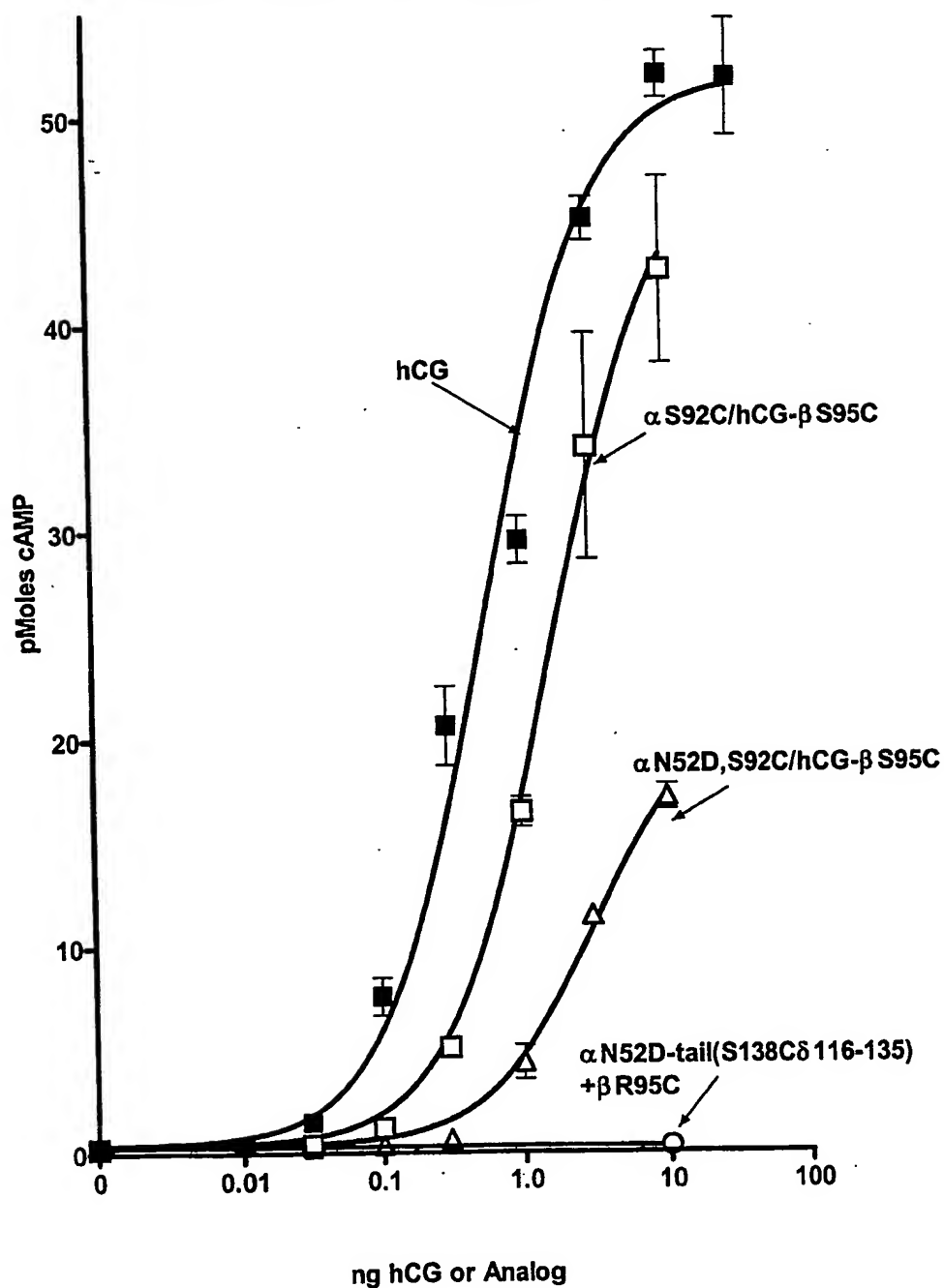


Figure 32

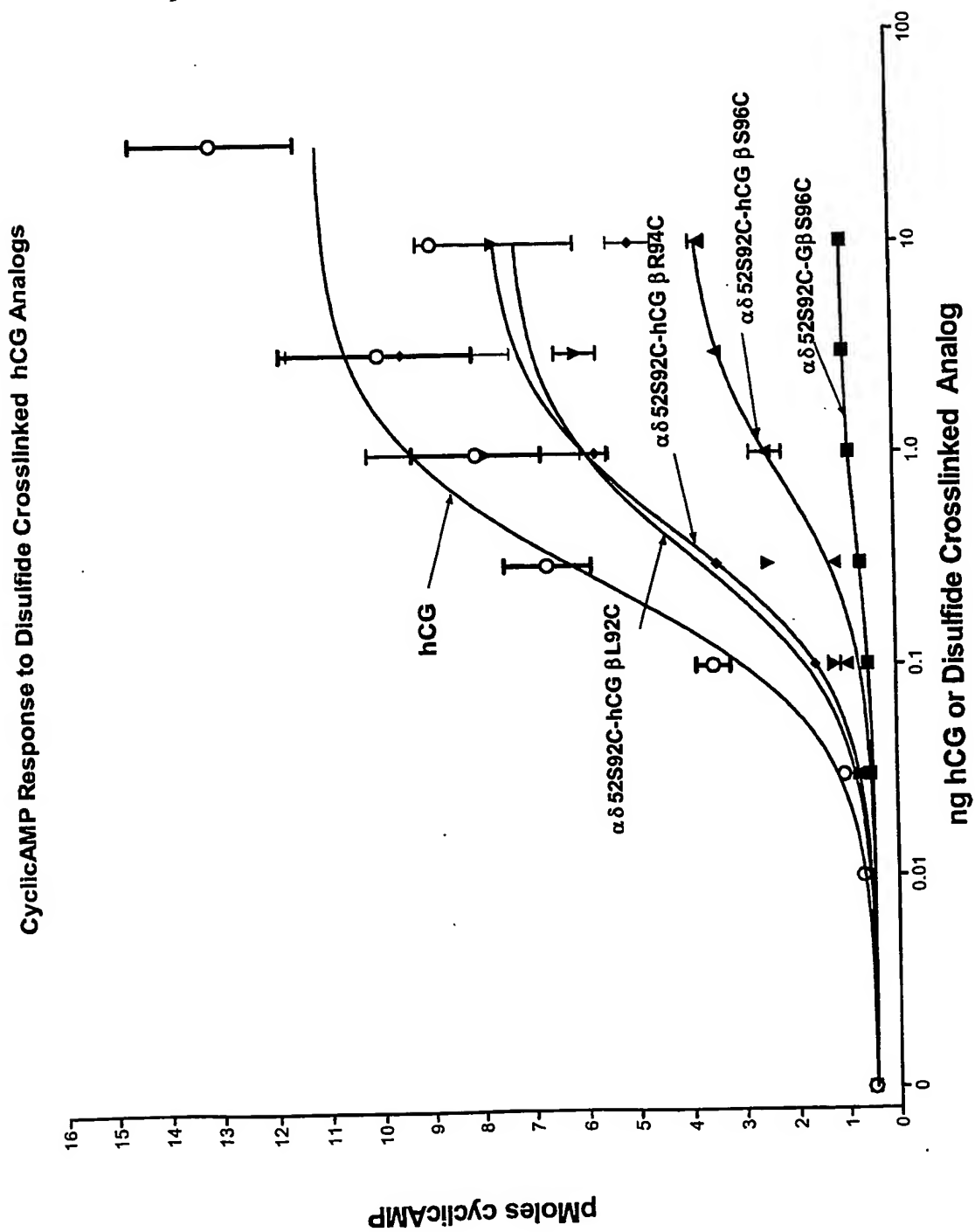


Figure 33